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THE LIFETIME GROWTH AND REPRODUCTION OF SELECTED STRAINS OF MICE

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1. INTRODUCTION

Most experiments in quantitative genetics perforce emphasise progress per unit of time, with the resultant quick turnover of generations. Thus, for instance, mouse selection experiments are commonly based on the use of first litters only, where nothing is known of the potential lifetime performance of the animals. Indeed, such information is probably irrelevant from the restricted point of view of a particular study. Yet, it is important to know how experimental procedures with the concomitant changes in genic arrays affect the natural fitness of the organism. In so far as such procedures may disturb gene frequencies in a population at equilibrium, fitness may be expected to decline, on the model of genetic homeostasis proposed by Lerner (1954). It is possible therefore that selection for any character in either direction may lead to a decline in natural fitness. Natural fitness of course has many components whose individual identities must often be obscure. Cumulatively, however, they are perhaps most clearly related to the total reproductive capacity of the organism, measured over the organism's lifetime.

The present study, which is essentially descriptive in nature, comprises a limited examination of these questions. Samples of mouse strains selected for high and low weight were placed aside and allowed to complete their reproductive life. Two main points were examined:

1. The effect of selection for six-week weight on subsequent growth, which may determine or at least affect reproductive capacity.
2. The effect of selection for weight on various aspects of longevity, and particularly on the total number of progeny weaned.

2. MATERIAL

Six groups of mice were employed in a comparative study. The designation and derivation of the groups were as follows.

1. RCL, a cross between Goodale's and MacArthur's large mice, and selected further for high six-week weight for 10 generations. The origin of the stock is described more fully by Falconer and King (1953).
2. MS, MacArthur's small mice, selected further in this laboratory for low six-week weight for 17 generations.
3. MXR, the F_1 generation of a cross between MS and RCL; reciprocal crosses are represented equally in this group.

4. NF, selected for high six-week weight for 27 generations.
5. NS, selected for low six-week weight for 22 generations.
6. NC, an unselected control stock from the same foundation as NF and NS, kept for 12 generations when this study started.

The last three stocks are described in greater detail by Falconer (1953).

Ten pair matings of each kind were set up and records of weight and litter production were kept. When an animal died, a replacement was provided in order to obtain records from the surviving member of the pair. Death usually resulted from natural causes, although animals were killed if they were in obvious distress and death in any case seemed imminent.

Much of the material, by nature of its erratic reduction until only one animal remained, does not lend itself easily to statistical treatment. Attempts were made to overcome some of the difficulties by the use of various transformations, invariably without much success. Fortunately, however, the main conclusions are often self-evident from the raw data, the presentation of which alone then suffices.

3. RESULTS

(i) *Length of life*

The age at death in days was calculated for all animals. Five mice whose deaths were due to accidental causes have been excluded. The results are shown in table 1, with the appropriate analysis of variance in table 2.

TABLE 1

Mean age at death in days

Stock	Males	Females
RCL	474·2±37·2	283·8±29·7
MS	700·2±44·1	452·2±52·8
MXR	683·1±66·1	471·4±60·5
NF	759·3±62·9	730·6±75·2
NS	900·3±99·4	747·3±73·0
NC	492·7±78·9	545·1±80·9

TABLE 2

Analysis of variance of mean age at death

	df	SS	MS	*p*
Total . . .	114	7 190 069	...	...
Between sexes .	1	414 812	414 812	$<0{\cdot}01$
Between stocks .	5	2 277 620	455 524	$<0{\cdot}001$
Interaction . .	5	396 135	79 227	$>0{\cdot}05<0{\cdot}10$
Error . . .	103	4 101 502	39 820	

Among the stocks tested in this trial, there was significant variation between stocks around a mean lifespan of 603 days. Furthermore, under the conditions prevailing in mating cages, males were significantly longer-lived than females, the weighted difference being 128 days. There is some suggestion that this difference is not constant

from stock to stock, though the interaction mean square is but barely significant at the 10 per cent. level.

Two comparisons enable us to examine the effect on length of life of selection for body weight, that between RCL and MS and that between NF and NS. In both cases, small mice were longer-lived though not significantly so in the latter case. Unexpectedly, among the N stocks selection in either direction increased the lifespan. In the MXR stock, the character displayed considerable heterosis, which is perhaps to be expected. The crossbreds equal almost exactly the longer-lived parental stock. The longest-lived animal in the whole experiment was an NS male, who lived to 1330 days (3 years 8 months). Four animals of the NS stock, two males and two females, exceeded 1000 days.

(ii) *Patterns of growth*

All animals were weighed when weaned at three weeks, and then at six weeks which is the usual age at mating. Males of the N stocks were weighed again at nine weeks, and males of all stocks at twelve weeks. Thereafter all males were weighed at four-week intervals until death. After six weeks of age, females were weighed immediately the birth of a litter was recorded, to avoid the obvious variation due to pregnancy. This, however, meant that many females lived over long periods, especially towards the end of their life, without a weight being recorded.

As animals died, the mean weight for each stock became determined by successively smaller numbers as time progressed, until ultimately only one animal remained. For this reason, the results are presented in fig. 1 as the growth curves of individual male mice. The distribution of growth patterns between stocks is sufficiently distinct, compared to variation within a stock, for the main conclusions to be drawn without any statistical refinements. However, to compensate for any bias in the apparent trends due to a possible correlation between weight and life-span within a stock, cumulative growth curves for males of each stock are shown in fig. 2. These curves were drawn by accumulating the growth of survivors over successive time intervals and are therefore largely independent of the absolute weight of the survivors at the time. The curves in fig. 2 are discontinued when they become determined by fewer than three mice.

We shall not consider the effect of the selection on six-week weight, which is another topic, but rather the consequences of the selection on further growth. Considering initially the weights of males, it is seen that growth was not complete at six weeks, and that the differences between large and small stocks increased rather than diminished, at least for a while. It is at once apparent that selection on total growth up to six weeks has resulted in vastly different mature sizes. Further, inspection of the growth curves shows that even within a stock, there

existed a correlation between six-week weight and maximum weight. Yet, this is not the whole story. A comparison of the two large stocks (RCL and NF) shows that both attained the same mature weight

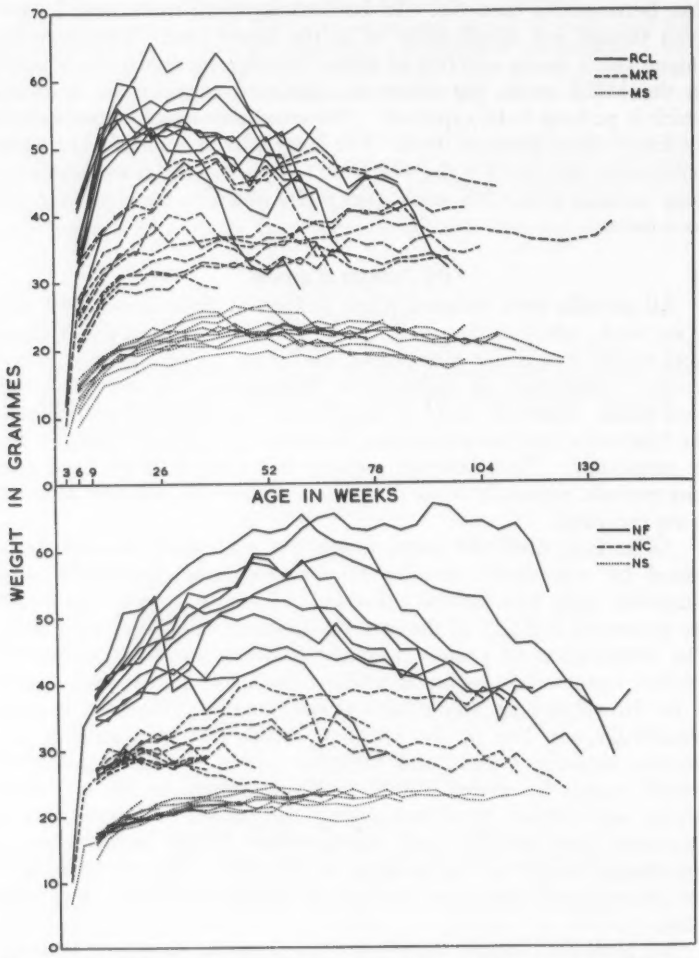

FIG. 1.—Individual growth curves of male mice, showing differences in growth pattern between stocks. (Five NS males lived longer than shown, but some of their weights inadvertently were not recorded.)

but at different ages, in the former at approximately six months of age, and in the latter at approximately one year. This is at least presumptive evidence that mature weight and the path whereby it is reached are to some degree under separate genetic control. On this

point, it can be noted that the RCL males reached their maximum weight at a much younger age than males of other stocks.

Once the maximum weight had been attained, a conspicuous difference in pattern emerged between the two heavy stocks and the others; the former, almost immediately and without exception, began to show a decline in weight which continued without arrest until death. The two light stocks and also the unselected and crossbred stocks, in contrast, showed no decline in weight and retained their

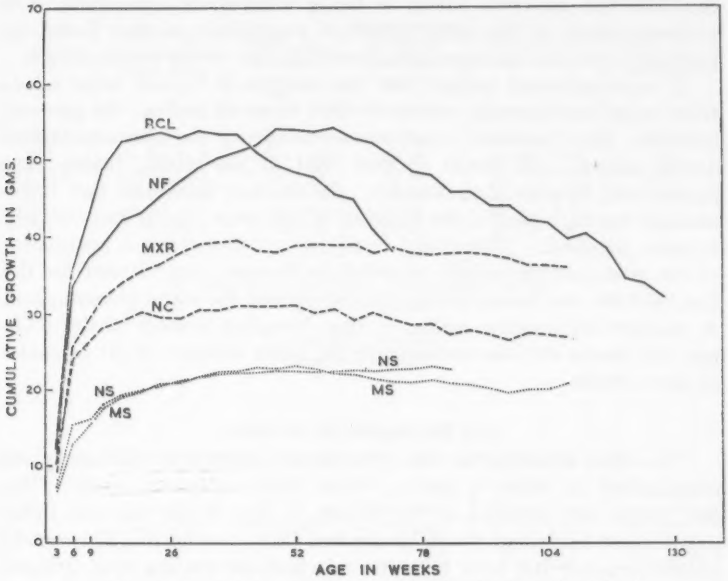

FIG. 2.—Cumulative growth curves of male mice for each stock (explanation in text).

maximum weight almost until death. It seems probable that this difference in weight pattern is related to the difference in fatness; the large mice contain much larger stores of fat, relative to body weight, than small mice (Fowler, 1958). The loss of weight of the large stocks in later life was probably due to the depletion of accumulated fat.

The crossbred stock (MXR) showed no evidence of heterosis with respect to mature weight. It did, however, maintain this weight, and it eventually exceeded the level of the heavier parental strain. Whether weight can be regarded as a heterotic character depends therefore on the age at which the character is measured; alternatively, one may argue that the nature of the "character"—in this case, weight—may alter, with age. For instance, it is quite possible that the MXR stock showed heterosis with respect to bodily dimensions at all ages, but failed to accumulate as much fat as the RCL stock.

The MXR group consisted of an equal number of the two reciprocal crosses. Whereas the group, as a group, was quite distinct from either of the two parental stocks, there was some evidence that within the group mice derived from RCL mothers were heavier than those derived from MS mothers. At maximum weight, the average difference was of the order of five grammes, and the two reciprocal types converged only trivially as they aged, indicating a permanent maternal effect on weight. Though the numbers are quite insufficient to establish this maternal effect as being statistically significant, it is proportionately of the same order of magnitude as that found by Brumby (1960) in analogous groups of mice at twelve weeks of age.

It was explained earlier that the weights of females were somewhat more ambiguously recorded than those of males. In general, however, they provided confirmatory evidence for the conclusions drawn above. All stocks showed that at six weeks, males were appreciably heavier than females. In the two light and two intermediate stocks, however, the ranking of the sexes almost immediately became reversed. This could easily be attributed to a peculiarity of the post-partum weight recorded in females, were it not for the fact that the two heavy stocks did not exhibit the same phenomenon. A possible explanation might be that breeding females of the RCL and NF stocks did not accumulate the same amount of fat as males of those stocks.

(iii) *Reproductive performance*

The data referring to the reproductive history of the stocks are summarised in tables 3 and 4, which refer to females only. The first point, not revealed in the tables, is that stocks did not differ significantly in the age at which the first litter was born. This would almost certainly not have been the case had not mating been delayed for all stocks until they were on average about eight weeks old. Having thus begun on an equal footing, four major factors govern the reproductive capacity of the stocks—the length of breeding life, the interval between litters, the average size of the litters at birth and the proportion of those born that survive to be weaned.

It is seen that the female mice in this trial stopped breeding at an average age of 300 days or so, but the variation among stocks is highly significant. Reproduction ceased at a younger age in the large stocks than in the small stocks. Further, referring back to table 1, some stocks, notably the NF, lived for a considerable time after reproduction had ceased. The correlation between the age at last litter and female longevity, with respect to stock means, is less than 0·5, despite an obvious causal relationship. However, this estimate, based on only six stocks, cannot be very accurate. Neither of the large stocks produced litters beyond the point when they would be expected to show the decline in weight characteristic of males of those stocks. Unfortunately, for reasons explained earlier, no weights

were obtained from females in later life to determine whether in fact they did lose weight.

The conclusions with respect to the age at which the last litter was born are clear. Firstly, selection for large size decreased the length of reproductive life while selection for small size increased it.

TABLE 3

Lifetime reproduction of females—numbers born alive

Stock	Mean age (in days) at last litter	Mean no. of litters	Mean interval (in days) between litters *	Mean total no. born	Mean litter size at birth †
RCL	201·2±30·9	3·4±0·92	42·65	24·2± 6·77	7·1
MS	295·8±51·2	10·6±1·49	22·74	49·0± 7·37	4·6
MXR	377·6±43·3	11·0±1·52	29·36	102·5±10·35	9·3
NF	238·4±28·9	5·4±0·83	34·44	33·0± 5·77	6·1
NS	428·0±44·7	11·9±1·47	30·92	48·8± 6·65	4·1
NC	307·2±27·1	8·0±0·54	32·00	44·8± 5·57	5·6

* $\frac{\text{Mean age at last litter—Mean age at mating}}{\text{Mean no. of litters}}$

† $\frac{\text{Mean total no. born}}{\text{Mean no. of litters}}$

TABLE 4

Lifetime reproduction of females—numbers and weight of offspring weaned

Stock	Mean total no. weaned	Proportion weaned	Mean total wt. (in gm.) of offspring weaned	Mean total wt. (in gm.) weaned by 183 days	Proportion of total wt. weaned by 183 days
RCL	18·6±5·70	0·77±0·027	198·99± 56·13	159·14±32·12	0·80
MS	31·1±6·30	0·63±0·022	205·70± 41·85	100·53±16·31	0·49
MXR	93·4±9·27	0·91±0·009	847·24±106·14	414·68±36·87	0·49
NF	18·8±4·57	0·57±0·027	176·54± 36·57	146·95±26·39	0·83
NS	37·5±7·98	0·77±0·018	265·45± 53·89	113·25±16·68	0·43
NC	33·4±5·16	0·75±0·020	298·31± 48·75	198·97±32·99	0·67

Secondly, reproductive longevity, as illustrated by the MXR stock, displays striking heterosis.

The length of reproductive life is reflected, to a large extent in the mean number of litters born to females of particular stocks. It is seen that the large stocks do not compensate for their shorter reproductive life by a more rapid litter production. On the contrary, the disparity between the large and the small stocks is magnified rather than diminished. However, the large stocks make up some leeway by producing larger litters, few though they may be. But the advantage of the small stocks in the number of litters born is a telling

one, and their superiority over the large stocks in the total number of offspring born is in no doubt.

The fitness of an animal depends of course not so much on the number of progeny to which it gives birth as on the number of those progeny that reach sexual maturity and themselves reproduce. However, it is common experience that relatively few losses occur in the laboratory mouse after weaning, and that sterility also is comparatively rare. The total number of offspring weaned therefore provides some assessment, though by no means an exact one, of the reproductive fitness of a mouse. When the appropriate column in table 4 is examined, the small stocks are seen to have retained their advantage in the total number of offspring by weaning time. It is further seen that this is mostly due to the initial advantage in number born, and that the variation in the proportion of offspring that survive to weaning is not correlated with large and small size. Perhaps the most impressive feature of the data is the striking heterosis displayed by the MXR stock, which weaned three times as many young as the better of its two parental stocks. The crossbred animals were very successful in all aspects of reproduction, and outstandingly so in the mean litter size at birth and in rearing those litters to weaning.

The pattern of reproduction in the mouse is well-known. The first litter on average is submaximal owing to fewer ova being shed. Litter size then remains at a fairly stable maximal level over three parities or more, but eventually it gradually declines. The decline is reported to be due at least in part to an increased incidence of foetal mortality (Hollander and Strong, 1950; Wanke, 1939; Murray, 1934). The mean litter sizes at birth of successive parities for each stock are shown in fig. 3. The graphs, which are discontinued when they become determined by fewer than four mice, all conform to the expected pattern. There is some variation in the parity at which the maximum litter size is achieved which, however, does not seem to be correlated with body size. The crossbred stock (MXR), despite retaining its superiority in mean number born over twelve litters, does ultimately fall to the level of the small stocks; in fact, its decline with advancing age is the most marked. Nevertheless, it appears that the onset of senescence, as judged by the decline in litter size with age, is somewhat delayed in the crossbred stock, a finding which fails to support a suggestion by Chai (1959) that hybridisation confers no such effect.

The general conclusion with regard to the reproduction of the stocks tested in this trial is that mice selected for small size have a far higher reproductive rate than mice selected for large size, and possibly a higher rate than the unselected control mice. It has further been shown that the advantage of small mice rests entirely on the number of litters that they produce, which in turn can be related to the length of reproductive life. The one crossbred stock tested displayed the expected heterosis in reproductive capacity.

(iv) *Total weight of offspring weaned*

Those concerned with the applied aspects of this study may legitimately ask whether the number of offspring weaned is as important as the total weight of those offspring, especially the total weight within a given time from mating. This is primarily an economic question for which there is no general answer, but the data collected in this study are presented in table 4. It is seen that the advantage

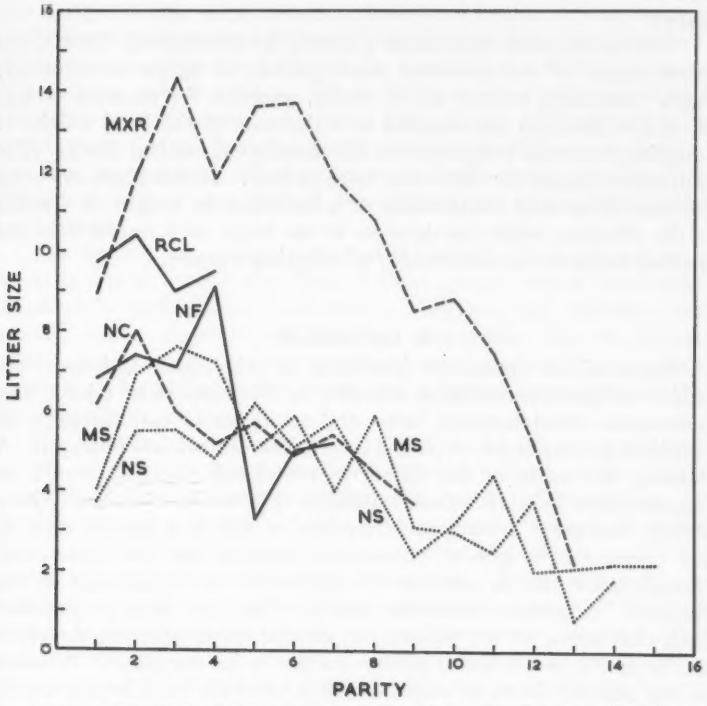

FIG. 3.—The relationship between litter size at birth and parity, for each stock.

of the small stocks in the total number weaned is sufficient to give them a superiority also in the total weight of these offspring at weaning. However, in neither of the two comparisons between large and small stocks is the difference statistically significant; in fact, the superiority of the MS stock over the RCL is quite trivial. Furthermore, as shown previously, the small stocks took a considerably longer time to accumulate those weights. For this reason, the cumulative weaning weights up to the age of 183 days (6 months) were examined. This period was chosen because by then all stocks are past their peak reproductive capacity, and are therefore at a stage when matings would be terminated under normal mouse management. The

appropriate column in table 4 shows the large stocks in a more favourable light. At this age, they exceed the performance of the small stocks, though not significantly. The conclusion is that if interest rests on the weight of offspring per female parent, stocks selected for high body weight are probably preferable to stocks selected for small size, which ultimately give more litters but these are smaller and contain lighter mice. A firmer conclusion would invoke economic considerations which are beyond the scope of this paper.

Two final points from table 4 should be mentioned. Firstly, the performance of the crossbred stock (MXR) is again outstandingly high. Secondly, among the N stocks, selection for six-week weight in either direction has resulted in a decrease of the total weight of offspring weaned, compared to the unselected control stock. The immediate reason for this is not hard to find. In the small stock the decrease is directly attributable to a reduction in weight as a result of the selection, while the decrease in the large stock results from the marked reduction in the number of offspring weaned.

4. DISCUSSION

None of the characters described in this paper had been the subject of previous artificial selection in these stocks of mice. The differences found between large and small stocks can therefore be classified as correlated responses to selection for six-week weight. A detailed discussion of the theory of correlated responses would be inappropriate here; a recent exposition is given by Falconer (1960). Briefly, however, a correlated response to selection cannot arise in the absence of a genetic correlation between the two characters, though other factors will also contribute to the magnitude of the response. A genetic correlation implies either that some genes affect both characters, *i.e.* are pleiotropic, or that genes affecting the characters separately are linked predominantly in the one phase. Whereas in the selected lines, it might be expected that equilibrium would have been reached between the coupling and repulsion phases, it might well be that genetic correlations as a result of linkage would be important in the crossbred stock examined in this study.

Selection on total growth up to six weeks has been shown in the previous section to have affected a number of other characters as well. Not surprisingly, perhaps, it has had a marked effect on subsequent growth and has led to different maximum weights, though the proportional differences between the large and small stocks are substantially the same at six weeks and at mature weight. From this alone, it would not be unreasonable to argue that the same genetic system controls both six-week and mature weights. But the different growth pattern of the two large stocks clearly suggests at least some genetic independence between growth rate and mature weight, and

thus shows broad agreement with the hypothesis of mammalian growth proposed by Dickinson (1960).

Selection for body weight had a marked effect on length of life and particularly on the length of reproductive life. The reason for this is obscure, but a possible parallel may be drawn from nutritional studies by McCay (1947), Ball *et al.* (1947) and Visscher *et al.* (1952). Briefly, these studies cumulatively show from work with rodents that the restriction of calorie intake through the diet lengthens life and also delays the age of reproductive failure, as judged by the capacity of the females to produce litters. Now, the parallel is pertinent only if the small stocks in this study can be regarded as a biological means of restricting calorie intake. For instance, small mice may utilise a relatively greater portion of their calorie intake to maintain body temperature. If so, then the direct cause of the differences found in length of reproductive life becomes a mechanical one resulting from differences in body size, though the complexities at the physiological level remain.

The finding concerning the relative longevity of large and small stocks is not in accord with that of Chai (1959), whose large strain significantly outlived his small strain. His material, however, was inbred, and is therefore not directly comparable with the present study. Chai found too that a hybrid stock derived from a cross between his large and small strains had a lifespan in excess of either parental strain.

The correlated responses to selection found in this trial were usually in opposite directions in the large and small stocks. On a homeostatic model, discussed earlier, this is not the expectation for characters related to fitness. The general finding suggests therefore that in the material examined here, genetic homeostasis was not a predominating feature of the base populations. There were only two instances in which the correlated responses assumed the same direction in both large and small mice, the comparisons being of course limited to the N stocks which contained a control. Firstly, the mean age at death increased in both selected stocks. The reason for this is obscure. It should, however, be noted that the age at which reproduction ceased, a more direct component of fitness, responded differently in large and small mice. Secondly, the total weight of offspring weaned declined in both selected lines for reasons given earlier. While this character is almost certainly related to some aspects of fitness, it is less obviously so than the total number weaned, where the correlated responses were in opposite directions.

The final conclusion from this study is that selection for a rapid early growth had an adverse effect on reproductive fitness, as judged by the total number of offspring weaned over a lifetime. This reduction in number of offspring resulted mainly from a drastic shortening of the length of reproductive life. Given time, mice selected in the opposite direction showed no reduction in number weaned, though of

course the total weight of offspring was reduced. In terms of practical application to domestic livestock, a good growth rate and high reproductive capacity are frequently twin objectives. The general conclusion is therefore discouraging. However, depending upon economic and biological considerations, the consequences of a shortened reproductive life in some species may not be serious. Further, the apparent negative correlation between growth rate and reproductive capacity may not be so great that its effect could not be overcome or at least diminished by appropriate selection techniques. And lastly, this study confirms that the crossing of suitably selected strains may offer a solution to what might otherwise become stagnating fertility problems.

5. SUMMARY

1. This paper reports the effect of selection for six-week weight in mice on their subsequent growth and on various aspects of their longevity, particularly on the length of reproductive life.

2. The material consisted of ten pair matings each of two large strains, two small strains, a large × small cross and an unselected control strain.

3. The average length of life over all stocks was 1 year 8 months. The mean life span of the small strains exceeded that of the large strains by approximately 6 months, while the crossbred stock equalled almost exactly the better parental strain.

4. The difference in weight between the large and small strains magnified with age though the proportionate difference remained fairly stable. However, the two large strains, once their maximum weight had been achieved, showed a decline in weight which continued until ultimately they fell to the level of the intermediate strains. The cause of the decline, which was not a feature of the other four groups, was probably the depletion of fat reserves.

5. The large strains had a short reproductive life, producing on average only $4\frac{1}{2}$ litters, against 11 or so in the small strains. On account of this the small strains eventually weaned almost twice as many offspring as the large strains.

6. Perhaps the most striking feature of the data was the heterosis displayed by the crossbred stock with respect to reproductive capacity. Compared to the better parental strain, the crossbred stock weaned three times as many offspring whose total weaning weight was four times as great.

7. The findings are discussed in relation to the original selection for high and low six-week weight.

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I wish to thank Mr E. D. Roberts for drawing the figures; I appreciate his patience with the cumbersome fig. 1.

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THE CONTROL OF INTRANUCLEAR DISTRIBUTION OF CHIASMATA IN *DELPHINIUM*

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1. INTRODUCTION

Although the inter-bivalent distribution of chiasmata has not been studied as extensively as the intra-bivalent, evidence has been available for a long time about its variable nature. Thus, Mather (1936) concluded, following chiasma frequency analysis in twenty seven different organisms, that negatively correlated chiasma frequencies occur in the two or more groups of chromosomes in the nuclei of a number of species but not of all. The non-universal character of this type of chiasmata distribution was also indicated by the observation that while some individuals of a species show negatively correlated chiasma frequencies, others may fail to do so and a further group shows them with respect to some bivalents only. The present analysis in *Delphinium* was undertaken after evidence had been obtained in the course of an earlier investigation on this plant, that experimentally induced changes in the number of chiasmata in two large bivalents, affect their frequency in the six small bivalents (Jain and Bose, 1960). This was observed in a number of plants analysed and it was felt that further studies on this material might prove to be of interest in relation to the above problem.

2. MATERIAL

Plants of *Delphinium ajacis* var. "Comet", a winter annual with a diploid chromosome number of 16 were grown as controls to colchicine induced tetraploids. Flower buds from five of these diploid plants were fixed in Carnoy to which a few drops of aqueous ferric chloride solution (1 per cent.) had been added for mordanting. The chiasma frequency scoring for detailed analysis was done at metaphase in temporary acetocarmine preparations. Pollen mother cells at early diakinesis when the chiasmata were not liable to any confusion, were also scored to find whether or not the two sets of observations show the same trend. Of the eight chromosome pairs in *Delphinium*, all of which are of fairly large size, two are considerably larger than the other six (figs. 1, 2) and this easy separation of the bivalents into two groups as well as the fact that a large number of cells can be easily analysed, makes the plant of considerable value for studies on inter-chromosome distribution of chiasmata. On the other hand, unlike organisms, such as *Vicia*, there is terminalisation of chiasmata in *Delphinium* as prophase advances and it has been supposed on the basis of considerations pointed out in the following section, that metaphase scoring gives results comparable to those at diplotene.

3. METHOD

In order to find out whether the chiasma frequencies in the large and small groups of chromosomes show any correlation, the method described by Elliott (1958) has been followed. This method involves the determination of inter-nuclear

variance which measures the variation in the total number of chiasmata among the pollen mother cells, and inherent variance which measures the variation in the distribution of chiasmata between the bivalents of the individual cells. If the inter-nuclear variance exceeds the inherent variance, a positive correlation is suggested. A negative correlation is indicated, on the other hand, if the inherent variance is greater than the inter-nuclear variance. As all the eight bivalents in *Delphinium* cannot be distinguished from one another—they can be separated into only two distinct groups—the inherent variance has been calculated to measure the variation in the distribution of chiasmata within the large and the small chromosome groups. The components of variation for n nuclei in *Delphinium*, then, are as follows :

Source of variation	Degrees of freedom
Total	$2n-1$
Between nuclei (inter-nuclear) . .	$n-1$
Between groups of bivalents . . .	1
Inherent (between groups within nuclei) .	$1(n-1)$

The last-named component has been designated as the inherent variance for the purpose of the present analysis. Correlation coefficients for the two groups of chromosomes were calculated employing the standard formula :

$$r = \frac{\mathrm{Cov}_{xy}}{\sqrt{(\mathrm{Variance}_x)(\mathrm{Variance}_y)}}$$

where x and y refer to chiasma frequencies in two groups.

4. RESULTS

(i) *Chiasma frequencies, inter-nuclear and inherent variances*

The observations on mean chiasma frequencies of each of the five plants are summarised in table 1. Also given in this table are the corresponding inter-nuclear and inherent variances.

The observations in table 1 show that at both metaphase and early diakinesis, inherent variance exceeds inter-nuclear variance. This is true for all the five plants and the difference has been found to be statistically significant in the case of four of them, the first three plants showing this significant difference both at metaphase and diakinesis. The analysis at the two stages thus provides evidence for a negative correlation between the chiasmata in the large and the small groups of bivalents. The fact that early diakinesis and metaphase observations show the same trend makes it possible to suppose that terminalisation does not significantly change the earlier condition with regard to the intra-nuclear distribution of chiasmata. Such an alteration would occur if the process of terminalisation in different bivalents is negatively correlated. As pointed out by Mather (*loc. cit.*), it is difficult to imagine such a correlation, particularly when terminalisation is complete. Hardly any interstitial chiasmata were found in the metaphase bivalents in the present material.

An examination of the observations in table 1 also shows that the negative correlation occurs in the case of plants showing relatively high mean chiasma frequency as well as others showing lower mean values and thus appears to be independent of such values. A t test, performed for their metaphase mean values showed that plants 1

and 2 and 2 and 3 have significantly different frequencies. Also, a test of heterogeneity of variances (table 1A) shows that the five plants differ in their inter-nuclear variance but not in inherent variance. In spite of differences in their inter-nuclear variance, however, all the five plants give indication of a negative inter-group correlation.

TABLE 1

Analysis of variance of chiasma frequency

Plant no.	No. of cells	Mean Xta per cell	Inter-nuclear variance	Inherent variance	Correlation
		(Metaphase)			
1	100	10·16	0·0953	0·1585	— *
2	100	9·69	0·0522	0·1569	— *
3	100	10·27	0·0770	0·1651	— *
4	100	10·83	0·1188	0·1415	— *
5	100	9·92	0·0952	0·1842	— *
		(Early diakinesis)			
1	50	12·22	0·1239	0·4067	— *
2	50	12·10	0·1237	0·2993	— *
3	50	12·18	0·1362	0·3429	— *
4	50	12·30	0·2104	0·4340	— *
5	50	12·22	0·2158	0·2673	— †

* Correlation negative, significant at 5 per cent. level.
† Correlation, not significant.

TABLE 1A

Test of heterogeneity of variances in table 1

	Inter-nuclear variance			Inherent variance		
	χ^2	D.f.	P	χ^2	D.f.	P
Metaphase .	16·9883	4	0·01 – 0·001	2·2934	4	0·7 – 0·5
Diakinesis . .	7·9305	4	0·1 – 0·05	3·9278	4	0·5 – 0·3

This shows that significant heterogeneity among inter-nuclear variances does not necessarily determine the direction of correlations as was suggested by the observations of Elliott (1958) on *Endymion*. The existence of large inter-nuclear variances in several of the plants, on the other hand, suggests the need for extending the analysis to the level of individual cells within a plant.

(ii) *Intra-plant, inter-cell variation in chiasma frequency and inherent variance*

The observations on the chiasma frequency described above are presented in a different manner to show the intra-plant, inter-cell distributions.

These distributions make it clear that the different pollen mother

TABLE 2

Intra-plant inter-cell variation in the distribution of chiasmata in large and small group bivalents at metaphase

Plant no.	No. of chiasmata in large bivalents	No. of chiasmata in small bivalents				
		5	6	7	8	9
		No. of cells showing stated chiasma frequency in the two groups of bivalents				
1	6	...	1	...	...	...
	5	...	8	3	...	...
	4	2	41	14	2	...
	3	...	10	10	3	...
	2	...	4	1	1	...
2	4	...	32	7	...	...
	3	...	34	20	2	...
	2	...	1	4	...	...
3	5	...	4	1	...	...
	4	...	33	24	3	...
	3	...	13	16	4	1
	2	...	1	...	...	...
4	6	...	1	...	...	...
	5	...	7	6	1	1
	4	...	30	29	12	2
	3	...	6	3	2	...
5	6	...	1	1	...	...
	5	...	3	2	...	...
	4	...	38	11	1	...
	3	...	34	6	2	...
	2	...	...	1	...	...

cells of a plant can be divided into several groups. This has been done by placing the cells showing the same number of chiasmata at metaphase in one separate group. Four such groups having cells with 9, 10, 11 and 12 chiasmata respectively have been thus obtained. Cells showing chiasma frequency beyond the range of 9-12 are very few in number (less than five) and have not been considered here. Fewer than five cells in any of the above four groups have also been discarded for the purpose of intra-plant analysis. The cells within each of the

four groups have been shown to exhibit no inter-nuclear variance and it has been of interest to see how the inherent variance varies in them. This inter-cell group variation in respect of inherent variance for the five plants taken both separately and collectively, is shown in table 3.

The results presented in table 3 show that with increase in the

TABLE 3

Intra-plant, inter-cell group variation in inherent variance

Plant no.	Inherent variance for different cell groups							
	Group 1 (9 Xta)		Group 2 (10 Xta)		Group 3 (11 Xta)		Group 4 (12 Xta)	
	No. of cells	Inherent variance	No. of cells	Inherent variance	No. of cells	Inherent variance	No. of cells	Inherent variance
1	13	0·1625	52	0·1468	25	0·2152	6	0·3148
2	38	0·0644	52	0·1608	9	0·1297	...	...
3	13	0·0000	49	0·1496	32	0·1804	5	0·3332
4	6	0·0000	33	0·0568	38	0·1368	19	0·2456
5	35	0·0190	44	0·0803	16	0·2194	...	...
1-5 Pooled	105	0·0503	230	0·1306	120	0·1923	30	0·3073

TABLE 3A

Test of heterogeneity of inherent variances in table 3

Plant no.	χ^2	D.f.	P
1	3·9899	3	0·3-0·2
2	8·1408	2	0·02-0·01
3 *	1·5277	2	0·5-0·3
4 *	12·8834	2	0·01-0·001
5	14·4739	2	0·01-< 0·001
1-5 Pooled	58·3599	3	0·01-< 0·001

* The cell groups with 0 inherent variance have been left out from the test of heterogeneity.

number of chiasmata in the cells, the inherent variance in most cases also increases. To see whether this apparent increase is statistically significant, Bartlett's test for heterogeneity of variances was performed for individual plants as well as the pooled data (table 3A). Inherent variances for different cell groups in plants 2, 4 and 5 are found to be heterogeneous, and so also the inherent variances in the case of pooled data which obviously has greater reliability as the variances were based on larger number of degrees of freedom. The general trend of observations on all the plants as well as the analysis of pooled data, leads to the conclusion, that the inherent variances in different cell

groups are positively correlated with their chiasma frequencies. The increase in the number of chiasmata is not expected, as such, to lead to as large variances as observed. The expected rate of increase in variance on a purely numerical basis, is the squared rate of increase in mean. Considering the two extreme cell groups 1 and 4, the expected increased variance of group 4 would be $12^2/9^2$ times variance of group 1, whereas in the case of pooled data, this increase is nearly six times. A considerable part of the observed increase must therefore be attributed to other factors. If it is assumed that competition leads to greater variance, the above results would show that the inter-bivalent competition for chiasmata for which evidence has already been presented, increases with increase in the number of chiasmata in a cell. The assumption is not without evidence, although it is derived from observations of a very different kind. Experimental findings of Sakai (1955) and Griffing (1953) on variances for quantitative characters in plants are compatible with this assumption.

(iii) *Intra-plant variation in correlation coefficients*

The effect of increase in chiasma frequency on intra-nuclear distribution of chiasmata can also be shown by calculating correlation coefficients of chiasmata in the two groups of bivalents. For this purpose, the pollen mother cells of each plant have been divided into two groups; cells with a chiasma frequency of more than 10 constituting the A group, those having 10 or fewer chiasmata the B group. The correlation coefficients for these two groups of cells in each of the five plants are given in table 4.

It is found that the negative correlation coefficient is stronger in the group A pollen mother cells in all the five plants, significantly so in three of them. If the inherent and inter-nuclear variances are determined for these two groups of cells, as has been done in table 5, it is observed that the inherent variance is greater in the A group cells than in the B group in four of the plants; in two of them, this is associated with an inter-nuclear variance which is smaller than the corresponding variance in the B group. The above results are thus in agreement with those presented earlier in showing greater variation in the distribution of chiasmata and greater inter-bivalent competition in cells with relatively higher number of chiasmata.

5. DISCUSSION

The genotypic control of chiasma frequency was demonstrated convincingly by Rees (1955) following his observations on crosses between inbred strains of rye. It has also been known, as pointed out earlier, that distribution of chiasmata in a plant may not be independently determined. More recently Rowlands (1958) has shown that in different plants of *Vicia faba*, there is a wide range of correlation coefficients, from negative correlations that are highly significant to positive which approach the level of significance. Variable

behaviour of this type has also been demonstrated by Elliott (1958) in *Endymion* and hyacinths. Both Rowlands and Elliott further found that the correlation coefficients are not related to mean chiasma frequencies in the plants. The present observations on *Delphinium* indicate that it is not possible to relate the inter-bivalent correlations with mean chiasma frequencies in the plants. The *Delphinium* results, however, are of further interest for two reasons. The fact that all the five plants show or tend to show correlation of the negative type

TABLE 4

Coefficients of correlation

Plant no.	Group A cells				Group B cells				t (1·96 at 5 per cent. level)
	No. of cells	Mean Xta	r	z	No. of cells	Mean Xta	r	z	
1	31	11·19	−0·8031	1·1066	69	9·69	−0·5172	0·5713	2·379
2	9	11·00	−0·9982	3·0000	91	9·56	−0·4913	0·5377	5·763
3	37	11·13	−0·8354	1·2056	63	9·76	−0·5618	0·6355	2·615
4	61	11·45	−0·5932	0·6826	39	9·38	−0·5263	0·5850	0·788
5	21	11·28	−0·7107	0·8886	79	9·55	−0·3633	0·3868	1·900

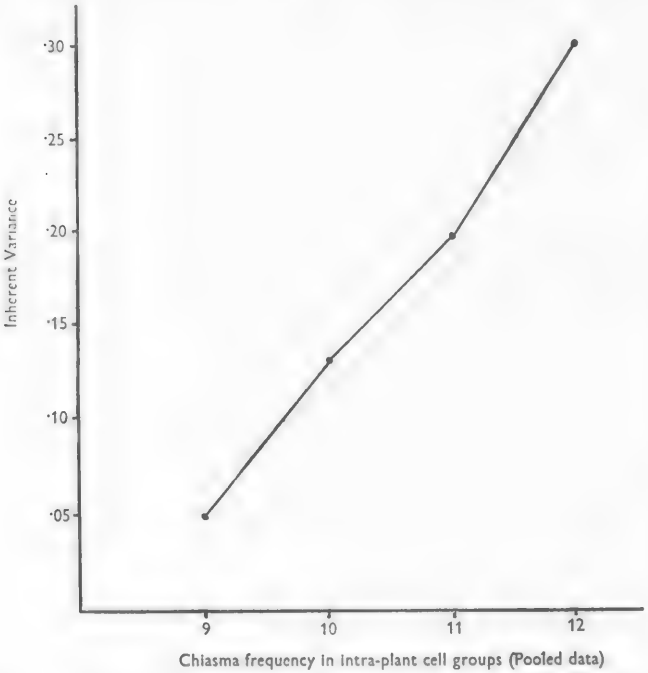
TABLE 5

Analysis of variance of chiasma frequency in the A and B groups of cells

Plant no.	Group A cells				Group B cells			
	No. of cells	Mean Xta	Inter-nuclear variance	Inherent variance	No. of cells	Mean Xta	Inter-nuclear variance	Inherent variance
1	31	11·19	0·0201	0·3009	69	9·69	0·0415	0·2072
2	9	11·00	0·0017	0·1511	91	9·56	0·0328	0·2699
3	37	11·13	0·0015	0·1787	63	9·76	0·0271	0·1480
4	61	11·45	0·0558	0·2069	39	9·38	0·0167	0·0760
5	21	11·28	0·0398	0·2946	79	9·55	0·0310	0·2012

contradicts the conclusion reached by Rowlands that such correlations are of little significance, being the occasional outcome of the mechanism controlling chiasma frequency. Secondly, the observations recorded on a large number of pollen mother cells in each of the five plants, make it possible to extend the analysis to intra-plant level and find out whether the negative correlation in individual cells is a function of the total number of chiasmata formed in them. The significantly greater inherent variances in most cell groups showing higher number of chiasmata clearly shows that the inter-bivalent distribution of chiasmata is affected by their number in a cell. A

similar conclusion has been described by Rees and Thompson (1956) on the basis of their detailed analysis of chiasma frequency variation in inbred and F_1 plants of rye. These authors found that bivalent variances and chiasma frequencies are negatively correlated between plants within inbred lines, but positively correlated within anthers of F_1 plants. The heterozygous *Delphinium* plants correspond more to the hybrids. Assuming that greater variance in *Delphinium* results from greater competition, the almost linear increase in inherent



TEXT-FIG. 3.—Variation in the inherent variance with chiasma frequency changes in the cells.

variance with increasing number of chiasmata (text-fig. 3) indicates that at lower levels of chiasma frequency, the inter-bivalent distribution of chiasmata is to a large extent independently determined. In other words, each of the bivalents is adapted to form a minimum number of chiasmata, beyond which competition sets in for the extra number of chiasmata, formed in the nucleus. Even if the above assumption is not made, the significantly greater correlation coefficient in the A group cells with their higher number of chiasmata would point to the same conclusion. A similar conclusion was reached earlier by Mather (*loc. cit.*) on the basis of observations of a different type made

Plate

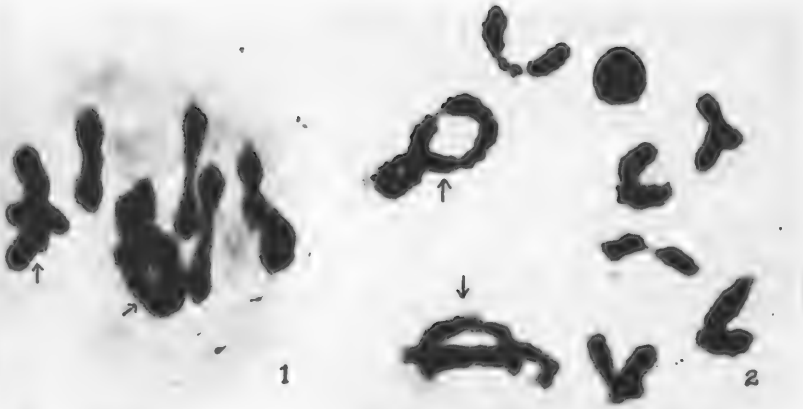


Fig. 1.—PMC showing the two large bivalents (arrowed) and the six small bivalents at metaphase, with a total of 3 and 8 chiasmata respectively. ×5500.

Fig. 2.—The two large bivalents, each with 3 chiasmata and the six small bivalents, each with 1 chiasma at diakinesis. ×5500.

by Lamm (1936) on rye plants. Lamm found that inbred rye with a low chiasma frequency fails to show inter-chromosome competition whereas ordinary rye with a higher chiasma frequency shows it often. Also, the recent finding of Wilson (1959) that experimentally induced changes in chiasma frequency in *Endymion*, affect the long chromosomes more than the short ones, is understandable on the basis of the above conclusion. It may be supposed that the induced changes affect that fraction of the chiasma frequency for which there is competition and the larger bivalents are better adapted to have it.

Mather has explained how, with bivalents in the same nucleus showing very different frequencies of chiasmata, there may exist differences in the competing ability of the different bivalents, which would explain the variable behaviour with regard to the intra-nuclear distribution of chiasmata in different organisms. It is also possible to offer an explanation for the failure, reported by Rowlands, to find any consistent relationship when the mean chiasma frequencies of a number of individuals belonging to the same species and their respective inherent variances or correlation coefficients are considered. The number of chiasmata beyond which competition occurs between the bivalents, may vary in individuals showing genotypically determined differences in their chiasma frequencies. Such a threshold variation would mean that a plant with relatively high mean chiasma frequency would not necessarily show a larger correlation coefficient.

The above interpretation of intra-nuclear distribution of chiasmata is obviously of interest in relation to the mechanism of chiasma formation. The fact that intra-nuclear chiasma distribution beyond a certain level tends to be determined by competition points to a regulating control which naturally discounts the suggestion that chiasma formation may have a purely mechanical basis. Such a distribution, suggesting as it does a limit to chiasma formation in the bivalents, has interesting genetical implications. It appears to be a manifestation of the control determining the amount of genetic recombination. The control of this recombination is of obvious importance in relation to stability on the one hand and continued adaptability on the other. The competition for chiasmata beyond a certain frequency would also possibly result in greater variation among the recombinants. It is not, however, possible to suggest that inter-bivalent competition for chiasmata has been selected to secure this purpose.

The earlier observations on this plant showing that experimentally induced reduction in chiasma frequency in one group of chromosomes leads to an increase in the number of chiasmata in the other group is also of interest, in relation to this point. It corresponds to the finding of Morgan *et al.* (1933) that prevention of crossing over on two of the long chromosomes of *Drosophila melanogaster* was accompanied by increased crossing over in the third chromosome, and emphasises the control of the amount of genetic recombination.

6. SUMMARY

1. Observations on the two groups of bivalents (large and small) in the pollen mother cells of five plants of *Delphinium* have indicated that their chiasma frequencies are negatively correlated.

2. It has been found that the inter-group correlation is stronger in cells with relatively higher number of chiasmata. The intra-plant analysis has also shown that when the different cell classes showing no inter-nuclear variance are considered, those with higher number of chiasmata show greater variation in the distribution of chiasmata among the two groups of bivalents.

3. The observations have been interpreted to indicate that at lower levels of chiasma frequency the intra-nuclear distribution of chiasmata among different bivalents is independently determined, but as the chiasma frequency rises, the bivalents show increasing competition.

4. The implication of these observations for the mechanism controlling chiasma formation has been briefly discussed. It has also been concluded that the inter-bivalent competition for chiasmata is a manifestation of the mechanism controlling amount of genetic recombination.

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GEOGRAPHICAL MELANISM IN THE LEPIDOPTERA OF SHETLAND

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1. FOREWORD

THE rapid spread of industrial melanism in the Lepidoptera which has taken place in the last 150 years has been a phenomenon unique in our knowledge and concept of biological changes. By experiment it has been shown that this spread is due in part to selective predation taking place in a changed environment (Kettlewell, 1956). This change has been brought about as an indirect effect of pollution fallout. The basic problem as to what part melanism has played in the past has, however, not been adequately answered. It is indeed surprising that melanic mutations seem to have arisen in so many species in so short a space of time, and even more so that the majority have had nearly complete dominance at inception. This suggests that in the evolution of melanism a preadapted genotype may have played a considerable part from time to time in the past history of those species manifesting it. Melanism may in fact confer advantages under a wide range of situations and certainly under circumstances very different from those in which the present-day industrial melanics are spreading. Some of these advantages may in part be due to the physical differences between black and white bodies.

It seemed possible, therefore, that field studies on the origin of non-industrial melanism might, at the same time, contribute to the larger subject of the origin and maintenance of polymorphism in general, about which there is at present so much speculation. A simple dimorphism involving the substitution of one colour form by another might be easier to study in the wild than those in more complicated situations.

It is a fact that although much has been written recently on the theory of polymorphisms (Williamson, 1958) and also on their genetical control, so far little experimental work has been undertaken in the field. Outstanding exceptions include the studies on the moth *Panaxia dominula* L. (Fisher and Ford, 1947), on the snail *Cepea nemoralis* L. (Cain and Sheppard, 1950), on the dimorphic females of *Colias* (Lep.) (Hovanitz, 1943), and on changes in the inversion frequencies in wild populations of *Drosophila* (Dobzhansky, 1959). Studies such as these, combined with laboratory investigations, have revealed basic mechanisms at work in the evolution of polymorphisms, such as disruptive selection to fulfil two optima (Mather, 1955; Thoday and Boam, 1959); the formation of the super-gene in mimicry and the simple

switch mechanism controlling it (Clarke and Sheppard, 1960; Ford, 1953); the advantages of heterosis (Haldane, 1955, 1956) and the breakdown and build up of dominance (Kettlewell, 1958*a*).

It therefore seemed essential to attempt to analyse non-industrial melanism in a variety of situations and to try to determine the relative advantages and disadvantages of the melanic mutants under the prevailing conditions, and at the same time to study the underlying mechanisms responsible for maintaining these ancient polymorphisms.

2. DISTRIBUTION OF NON-INDUSTRIAL MELANISM IN BRITAIN

Out of approximately 600 species of macro-Lepidoptera which occur in the southern half of Britain, probably fewer than 10 maintain melanic polymorphisms in non-industrial areas. These are now confined to Wales, Devon, Cornwall and parts of other southern counties to the west of Surrey. The whole of eastern England must be considered as being affected by pollution fallout, the effects of which must overshadow long existing polymorphisms.

Non-industrial melanism is also found rather abruptly on the western coastlines, notably of Cornwall and to an even greater extent of western Ireland.

Melanism was also known to occur in other more specialised biotopes. It was established, for instance, that a number of species resident in relict Caledonian pine forests were polymorphic for melanism and that in *Cleora repandata* Cl., the only one so far investigated, there was a one-gene difference between these two forms, the melanic form being dominant to the typical. Though probably of great antiquity, it is phenotypically similar to the Industrial Melanic mutant which is now found widespread throughout central industrial England. Furthermore, evidence was provided that each allele had both advantages and disadvantages in these ancient pine forests and that the result was a balanced polymorphism (Kettlewell, 1957). Situations such as these no doubt today reflect local opportunities for the survival of melanic polymorphisms, but it is likely that in the past they were much more widespread. There exists, however, one major factor to be considered. Between latitude 50° and latitude 60° there is a correlation between the occurrence of melanism and the distance northwards. This is evident in the North American Continent where an examination of specimens in the American Museum of Natural History showed that the farther north samples were collected the higher both the frequency of melanic forms per species became and the number of species exhibiting melanism.

The same applies in Britain. The farther north one proceeds up to latitude 61° the more melanism is encountered, so that in Scotland it occurs in many species, both on the coast and inland. To the north of the Scottish mainland lie the Orkneys, Shetland and, farther

north still, the Faeroes. In all these groups of islands melanic forms occur. It was decided, therefore, to investigate non-industrial melanism in one of these isolated areas.

3. MELANISM IN THE SHETLAND ISLES

It appeared to me that the Shetland Isles, where melanic forms were known to be common, would be the ideal place for a fuller investigation into non-industrial melanism. Here there could be no question of pollution fallout, and here the countryside has remained comparatively undisturbed by Man. During the last Ice Age (40,000 B.C. to 18,000 B.C.) it is likely that much of Shetland was not covered by ice, nor is it certain that it was covered during the last but one Ice Age (Strathmore) approximately 100,000 years ago. If this is so, it is possible that some of the fauna may be very much older than previously believed.

We have undertaken two expeditions to the Shetland Isles, in 1959 and 1960. Of the 78 species of moths recorded from these islands, 15 are migratory and are therefore unlikely to have acquired local forms. Migration may put a species at a disadvantage on the periphery of its range, in that an advantageous gene-complex, built up over previous years to adjust to local conditions, may be destroyed by an influx of fresh individuals from another environment (Haldane, 1956). Of the 62 native species unaffected by migrant individuals 27 have produced local forms, and of these one-third are melanic. These may be classified into three groups:

1. Species in which 100 per cent. of their population is melanic. They have discarded their normal alleles found elsewhere.
2. Species in which the two forms co-exist as a balanced polymorphism.
3. Species in which there is found an indefinite range of forms from black to pale; these presumably reflect either a multifactorial situation, or one in which variable heterozygotes occur.

The most obvious environmental change at these latitudes is the greatly diminished number of hours of darkness during summer and, what is probably more important, the long periods of twilight. These coincide with the warmest months in the year, when the majority of moth species appear as adults. This necessitates their taking flight in daylight. Under these conditions light coloured insects, which farther south have the protection of darkness, are more conspicuous than dark ones, so that extensive predation could take place if predators were available. We are able to provide evidence that in Shetland bird predators are extremely common, particularly in the north, and furthermore that they feed on moths both on the wing and on the ground. Farther north, however (above latitude 61°), melanic polymorphisms are less frequent. In the Faeroes, of the 23 indigenous

species (Wolff, 1929) only 4 (23 per cent.) show melanism and in Iceland 2 (10 per cent.) out of 21. This may reflect a relaxation of bird predation farther north, though this is unlikely to be the only reason.

Examples of melanic Lepidoptera found in Shetland are shown on plate I. Figs. 1 and 2 depict the English and Shetland forms of *Diarsia festiva*, Schiff. respectively, the latter being referred to as ssp. *thulei*, Staud. This race is distinct in appearance from *f. typica* of the British mainland, being darker and smaller, and falls into group 1 of the classification. We have bred the larvæ from ova, and we find that they bear little resemblance to those of *festiva f. typica*. One of our team, C. J. Cadbury, has succeeded in crossing the two races. The majority of larvæ and the single moth bred from them were intermediate in appearance between the parental races. It is likely that several genes are involved in the differences: ssp. *thulei* seems, in fact, to be well on the way to speciation. It is a good example of group 1 of our classification. Other species which are in a similar position are *Eupithecia venosata*, Fabr. and *Hadena conspersa*, Esp. (figs. 3-4). Both these feed exclusively in the Shetlands on *Silene maritima*, the Sea Campion, and are therefore limited to the shoreline. Though in Shetland considerable variability occurs in both these species, examples are never as light as the *f. typica* found elsewhere.

Species showing a clear-cut polymorphism (group 2) with distinct phenotypes suggesting disruptive selection are uncommon. However, one of the most successful of species in the Shetlands, *Amathes glareosa*, (Esper, 1788), which is to be discussed in considerable detail in the following two papers, falls into this category (plate II, fig. 1). It will be noted that *f. typica* has little resemblance to the black *f. edda* (Staudinger, 1891). *Lygris populata* L. should be relegated to the same group with its black *f. masanaria*, Freyer, but in this species the melanic is variable (plate I, figs. 5-6). *Amathes xanthographa*, Fabr. (plate I, figs. 7 and 8) should also be included in group 2.

Group 3, in which an indefinite range from pale to dark forms occurs, contains the majority of species. Amongst these are the following: *Entephria cæsiata* Schiff. (plate I, figs. 9 and 10), *Xanthorhoë fluctuata* L. (plate 1, figs. 11 and 12) and *Hepialus humuli* L. (plate I, figs. 13 and 14). The Shetland melanic form of *E. cæsiata*, ab. *atrata* Lange, is phenotypically identical to the industrial melanic form found near Paisley in Scotland, and specimens from both localities are referred to by this name.

The genetic control of melanism found in a similar situation to that of some species in Shetland has been analysed by Ford (1953). *F. curtisii* of *Triphæna comes* L. which occurs in North Scotland, is controlled by a major gene which has incomplete dominance. Ford demonstrated that the degree of expression of melanism was materially affected by the constitution of the gene-complex. He showed that when *curtisii* from the island of Barra was out-crossed into a gene-complex from Orkney (with which it could have had no recent contact)

the heterozygote *curtisii* were much more variable. Whereas the phenotypes had segregated in his initial wild stock, there was now a complete gradation from black to light. This demonstrated that the melanism of *f. curtisii* depends on one major gene which, however, expresses itself differently according to the modifying genes present.

This grouping of non-industrial melanism under three headings must therefore be considered arbitrary. Though others might place certain species in a different category, it is a convenient method of classification as a first step to understanding the origin of melanic polymorphism in Shetland. The classification into three groups reflects phenotypic expression which may bear no relationship to their genetic control.

4. PREVIOUS SHETLAND DATA

Not until the end of the last century was it realised that the Shetland insect fauna was very different from that found on the British mainland. At that time the interest was, as it still is to a large extent, in obtaining specimens for collections. Shetland offered few comforts then for collectors and was largely inaccessible. It is not surprising, therefore, that the few Lepidopterists who visited these islands were professional collectors, sent there to bring back as many aberrant specimens as possible, and paid by wealthy collectors who were not prepared to undergo the hardships themselves. A more important consideration is that the emphasis was therefore on extreme examples and that, as is the rule in all collections, the insects which were kept bore no relationship to their frequency in the populations from which they had been extracted. All normal specimens, similar to those found on the British mainland, were discarded. Violent frequency changes appeared in their yearly samples, and these were interpreted as reflecting the direct effect of seasonal differences on the insects themselves.

One of the first collectors to work in Shetland was McArthur, who visited the Mainland in 1880 and 1881 and subsequently Unst in 1883 (Weir, 1880, 1881 and 1884). He recorded a large number of melanic forms. The current explanation at this time was that melanism was the direct effect of humidity, and so it is not surprising to find that South (1893), who had never been to Shetland, decided that McArthur's melanic forms came from " boggy meadows ", whereas the more normal individuals were collected on drier cliffs and hillsides. More specifically he refers to *H. humuli* as behaving in this way. Similarly he appears to have thought that the dark form of *A. glareosa*, *f. edda*, was confined to " low-lying wet moors near Baltasound ". Other collectors about this same period were L. A. E. Sabine and Salvage, many of whose specimens can be found in the British Collection of Lepidoptera of the British Museum (Natural History) at Tring, but they published no records, presumably wishing to retain a financial monopoly.

Shetland has been visited on many occasions by collectors during the present century (Hare, 1957; Harper, 1958) but with the same object of procuring specimens for their own private collections only. On no occasion have frequencies of the forms been recorded. The reasons for melanism in the Shetland Lepidoptera have remained obscure.

5. CHOICE OF MATERIAL

In attempting to investigate and analyse the advantages and disadvantages of melanic forms in the wild, under the difficulties imposed by climate and lack of facilities, and without guidance from previous work, it is necessary to choose a subject which offers as few complications as possible. Easy recognition of the melanic form is therefore essential and also the choice of a species which is common, widespread and which could easily be collected. Mark-release-recapture experiments were envisaged in which the two contrasting forms would be released at the extremes of their frequencies. Furthermore, a clear-cut dimorphic species would be desirable, and one which would conform with the majority of situations found in industrial melanism.

For these reasons I decided to undertake a full investigation into the species *A. glareosa* Esp. and its dark form *edda*, Staud.

6. THE NATURAL HISTORY OF *AMATHES GLAREOSA* ESP.

(i) *Description of Morphs.*

With the exception of Shetland and the Orkneys, only the typical form of this species is found throughout its range (plate II, fig. 1). In this the ground colour is either light grey or light grey tinged with pink: pink forms are commoner in the southwest of England. Occasional specimens with a dark ground colour have in the past been recorded from parts of Yorkshire and also from Perthshire (Barrett, 1897). In Shetland *f. typica* is more usually of a slate-grey colour. On each forewing there are two conspicuous black wedge-shaped marks with their broad bases lying towards the costa. This pattern is without doubt disruptive. The efficiency of its patterning must therefore vary according to the depth of vegetation and the angle and brilliance of the sun. In common with most Noctuid moths the forewings alone are visible when at rest, and these marks serve to break down the insects' outline in the shadows cast by overlying foliage (plate II, fig. 2).

The melanic form *edda* was first discovered by McArthur in 1883 (Weir, 1884). It is found only in Shetland, including Fair Isle, and the Orkneys: *A. glareosa* does not occur in the Faeroes. *F. edda* bears little resemblance to *f. typica*. It is of a dark brown-black colour with light orbicular and reniform outlines on the forewings. The thorax is, however, greyish in many specimens. It is important to note that

the hindwings, which are only exposed in flight, are of a light colour. A further observation is that not only is the dorsal surface of the abdomen black, but also the ventral. This is an unusual occurrence in melanic Lepidoptera that have light-coloured hindwings, and must have a survival value. The significance of this will be discussed later. In its normal resting position on a peaty background *f. edda* is most difficult to see (plate II, fig. 3). This form therefore reflects the substitution of a disruptive pattern by a highly efficient cryptic one when resting on peat.

(ii) *Habits of Imago*

The species begins to emerge in the first week of August and in Shetland has a peak period in about the third week. By the end of the first week in September it is becoming scarce. On the British mainland, however, the period of emergence is longer, and I have taken a specimen in Cornwall as late as early October. The moth spends the day motionless on the surface of the ground beneath heather, but on wet days I have observed that it runs under small stones and then takes up a position so that only its head and thorax are visible. It flies at dusk, and the maximum flight period is probably half an hour later. It is attracted freely to light, particularly ultra-violet. It visits flowers regularly, especially heather, but in the south I have also taken it on ivy blossom. This species comes but reluctantly to artificial baits such as "sugaring mixtures". Mating occurs before midnight, almost certainly on the day of emergence, and pairs can occasionally be found *in copula* hanging from wire fences. Ovipositing most likely does not commence before the following night. In order to contribute to the next generation it is therefore necessary for the females to survive one period in daylight. This is a most important consideration. Most cryptic species hatch under cover of darkness, thereby avoiding immediate exposure on a possibly incorrect background. It has been pointed out that a species which hatches at dusk, pairs rapidly and succeeds in laying all its eggs on the first night would escape the consequence of visual predation, and that therefore colour and pattern would be of little consequence to survival (Kettlewell, 1958*b*). This is not the situation in *A. glareosa*, which we have shown may live up to two weeks in the wild, during the whole of which period ovipositing can be undertaken. We have observed that visual predation by birds takes place extensively. It is likely, therefore, that the two forms would have a different mortality rate due to selective predation and that this would vary according to the conditions under which they have to pass the day. It is in fact likely that this colour difference may play a major part in maintaining this polymorphism.

(iii) *Life history*

There is a one-year life-cycle, but it is possible that occasionally, in common with other northern species, a second winter may be passed in the pupal state. The moth lays its eggs singly, probably

on heather in the wild, but in captivity on heather, muslin or cardboard. They are spherical and ribbed and of a pale yellow colour. After a few days fertile ova can be recognised by flecks of red appearing around the upper pole. After two or three weeks the eggs darken and by the beginning of October all ova are blackish-grey. The following eight weeks is certainly a most vulnerable period in the life history of the species; in 1959, 100 per cent. of our stock died at this stage, and on dissection the eggs were found to contain fully formed larvæ. In 1960, by keeping ova cool and in a damp atmosphere, we succeeded in hatching a large proportion of them, but the period over which they hatched varied from 30 to 80 days, and within one brood (g/24/60) from 47 to 80 days. It seems in fact as though the larva goes into hibernation for a period within its eggshell. This is a well known phenomenon in certain species such as *Lasiocampa trifolii* L. Furthermore, in *A. glareosa*, no matter under what conditions the young larvæ were kept, a large proportion died in the first twenty-four hours after hatching. Although our broods were distributed to many of the best breeders in Britain, all had the same results. It is likely that a heavy mortality takes place in nature at this same period in the life history of the species. Those larvæ which survive the first forty-eight hours attain full growth with a comparatively low mortality rate. In warmth in the laboratory we accomplished this in under two months. In nature it is probable that the small larvæ feed slowly on warm nights throughout the winter. This much we know for certain; that full-grown larvæ have been found but rarely in the spring, and then only at night. They were feeding on plants such as hyacinth (Barrett, 1897). They pupate in April or May, and they do this in cocoons below the surface of the ground. Both larva and pupa are therefore largely protected from visual predation.

(iv) *Genetics of* A. glareosa f. typica *and* f. edda

F. edda has never previously been bred in the laboratory. *A. glareosa* is a very difficult species to rear in captivity; furthermore the larva has only rarely been found in the wild. We are at present engaged in breeding the offspring of 46 wild females. Table 1 gives a list of the wild caught female phenotypes along with their local gene frequencies and the number of imagines which have emerged up to date. From these data, particularly those of broods g/22/60 and g/14/60, it appears that *f. edda* is dominant to *f. typica*. *F. edda* varies from a light brown to black, but there is no obvious segregation within the *edda* of our broods. The females are usually darker than the males and this applies particularly to the hindwings. In the north of Shetland, where the heterozygotes must form approximately 12 per cent. of the population, the very black form is common and pale *edda* less so. In the south, however, where *edda* forms only 2 per cent. of the population, the black form is rare and nearly all the *edda* are less

extreme. We have further proof that these two forms are controlled genetically in that two of our party took a halved mosaic in south Shetland in which one side was largely *f. edda* and the other *f. typica*.

TABLE 1

Breeding results: Amathes glareosa, 1960-61

Brood no.	Phenotype of parent	Locality	Typica local gene-frequency %	Typica	Edda
g/37/60	typica	Unst	16·4	0	4
g/40/60	edda (light)	,,	16·4	0	3 (2 dark)
g/29/60	typica	*Mainland* Reawick	63·7	0	2
g/ 2/60	,,	Aith	66·3	5	7 (light)
g/31/60	edda	Skellister (S. Nesting)	61·6	3	1
g/13/60	,,	N. border of Tingwall Valley	86·9	1	1
g/19/60	,,	,, ,,	86·9	0	6
g/20/60	,,	,, ,,	86·9	1	2
g/21/60	,,	,, ,,	86·9	1	0
g/22/60	,,	,, ,,	86·9	0	12 (3 dark)
g/14/60	,,	S. border of Tingwall Valley	88·7	3	16 (2 dark)
g/15/60	,,	,, ,,	88·7	2	0
g/16/60	,,	,, ,,	88·7	1	1
g/ 8/60	,,	Boddam	99·0	0	2
g/ 9/60	,,	,,	99·0	0	1
g/11/60	,,	,,	99·0	1	0
			Total bred = 18		50

In the following two papers, the distribution of the two forms of *A. glareosa*, and also their differential survival and habit differences, are discussed.

7. SUMMARY

1. In previous papers I have produced evidence on the factors responsible for the rapid spread of industrial melanism in the Lepidoptera. I suggested that melanism might in the past have conferred advantages under conditions very different from those found today.

2. Evidence was provided that non-industrial melanism still occurs in the world today in widely different environments and that here we might discover the previous benefits conferred by melanism.

3. We have established that there is a correlation between the amount of melanism and the distance northwards between latitudes 50° and 60°.

4. In the Shetland Isles one-third of the moth species have melanic forms.

5. It was decided to investigate one species and attempt to analyse the advantages and disadvantages of its two morphs in a state of apparent long-standing balanced polymorphism.

6. The species selected was *Amathes glareosa* Esp. and its melanic form *edda*. The genetical control of the two forms, the life history and habits are discussed in this paper.

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Plate I

Shetland Lepidoptera with their melanic forms. · ¾.

Figs. 1 and 2.—*Diarsia festiva*. Schiff and ssp. *thulei*, Staud.
Figs. 3 and 4.—*Hadena conspersa*, Esp. and melanic Shetland form.
Figs. 5 and 6.—*Lygris populata*, L. and Shetland form *masanaria*.
Figs. 7 and 8.—*Amathes xanthographa* Fabr. and Shetland melanic form.
Figs. 9 and 10.—*Entephria cæsiata*, Schiff. and *f. atrata*, Lange.
Figs. 11 and 12.—*Xanthorhoë fluctuata*, L. and Shetland melanic form.
Figs. 13 and 14.—*Hepialus humuli*, L. and Shetland *f. Shetlandica*.

(*Photo J. Haywood, Oxford University*).

Plate II

FIG. 1.—*Amathes glareosa*, Esp. and its melanic *f. edda*, Stgr. Disruptive polymorphism. ×2.
(*Photo J. Haywood, Oxford University*).

FIG. 2.—*Amathes glareosa f. typica* at rest on ground, Unst 1960. ×3.
(*Photo H. B. D. K.*)

FIG. 3.—*Amathes glareosa f. edda* at rest on ground, Unst 1960. ×3.
(*Photo H. B. D. K.*)

THE STUDY OF A CLINE

AMATHES GLAREOSA ESP. AND ITS MELANIC *F. EDDA* STAUD (LEP.) IN SHETLAND

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1. INTRODUCTION

In the previous paper the occurrence of melanism in Lepidoptera in the Shetland Islands has been reviewed and the theoretical interest of studying the factors which contribute to the maintenance of melanic forms in so many species in this group has been set out (Kettlewell, 1961*a*). It has also been suggested that for practical reasons, the moth *Amathes glareosa* Esp. with its distinct melanic form *edda* would be suitable material for investigating the factors contributing to non-industrial melanism. This paper and the one that follows give an account of the work on this insect carried out in Shetland in the summers of 1959 and 1960.

Until recently knowledge relating to *A. glareosa* was limited to the casual observations of collectors, many of whom visited Unst, the northernmost main island of the Shetland group, for the specific purpose of catching (among other things) *f. edda*. This form of the moth was not known to occur elsewhere. *F. typica*, however, had been reported from Dunrossness on the south Shetland mainland. Hence the original problem was the relatively uncomplicated one of discovering why *A. glareosa* was largely replaced by a melanic form on Unst, an island 12 miles long by 6 wide. However, it soon became apparent that the situation was more complex than originally thought.

The first step consisted therefore of studying the structures of the two populations: at Baltasound on Unst in the north, and at Dunrossness in the south Shetland mainland. These preliminary investigations in 1959 were combined with a study of the natural history of the species. Dunrossness is only about 50 miles to the south of Baltasound as the crow flies, but here the population of the moth was thought to be entirely composed of the typical form similar to that found over the mainland of Britain. In 1959 we sampled the populations and made the discovery that while *f. edda* occupied a frequency of 97 per cent. ($\pm$0·34) at Baltasound (*f. edda* 2462: *f. typica* 77), it also occurred at a frequency of 3 per cent. at Dunrossness in the south (*f. edda* 3: *f. typica* 96). Moreover, a small sample of 23 moths caught at Hillswick, which is situated about midway between the two, showed that here the

FIG. 1.—A frequency map of *Amathes glareosa* and its melanic form *edda*, comprising 20,000 records from 22 localities in Shetland. The numbers refer to the locations listed in table 1.

population was 83 per cent. *f. edda* (*f. edda* 19: *f. typica* 4). Hence it was clear that the species seemed to be dimorphic throughout the length of Shetland, and our study was not merely concerned with the replacement of *f. typica* by *f. edda* over the limited area of Unst, but also with the co-existence of the two forms over the whole of the Shetland Isles.

The account that follows is of our investigation of the distribution of the frequencies throughout Shetland in 1960.

2. METHODS OF INVESTIGATION

The Shetland Islands are situated about one hundred miles to the north of the mainland of Scotland. The group is 54 miles in length from north to south and comprises three main islands, Unst, Yell and Shetland Mainland separated by two sounds, each approximately half a mile in width (see fig. 1). There are about a hundred smaller islands at distances of a few hundred yards to 27 miles from the main group (*viz.* Fair Isle). The plan was to investigate the composition of the populations at as many places as possible throughout the Shetland Isles. The minimum sample envisaged was fifty specimens from each place. Although the majority were considerably larger than this a few were smaller, and all except the smallest ones have been included in the results (table 1).

The technique employed was to take samples of the population by means of mercury vapour light traps. Whenever possible the electricity supply was taken from crofts, but when not available (neither Yell nor Fetlar have mains electricity), portable generators were used. It has been shown in the case of *Biston betularia* L. that both the typicals and the melanic mutants are attracted equally to light by comparing the proportions of those which were attracted to virgin females with those which came to light (Kettlewell, 1955). We had no reason to suppose that the two forms of *A. glareosa*, once they had taken flight, behaved otherwise.

It is perhaps worth emphasising a point already made, that the two forms of the moth are completely distinct, and there is rarely any difficulty in distinguishing the two (plate II, fig. 1, in Kettlewell, 1961*a*). The only times any doubts arose were with very worn specimens, but they could always be assigned to their correct phenotype by reference to the colour of the underside of the body. In regions where a low frequency of *f. edda* occurred, such as Dunrossness, south Shetland, many of the melanics were rather lighter and browner in wing colour and more variable in pattern than in places with a higher incidence. These specimens were almost certainly heterozygous for the *edda* gene, but can be referred to *f. edda* without hesitation. The Orkney and Fair Isle specimens fall into this same category where the *edda* frequencies (in small samples) are 15 per cent. (*edda* 3; *typica* 17) (Ffennell, 1961) and 27 per cent. (*edda* 15; *typica* 40) (Hardy, 1961) respectively. A few specimens caught on Shetland could not be properly classified as *f. typica* nor *f. edda*. As the appearance of these

specimens was fairly uniform among themselves, they may well represent another genotype. They have not so far been studied further.

Approximately 20,000 individuals were trapped and examined. The majority of these were caught at the two centres of the experimental work to be described in the following paper (Kettlewell, 1961*b*). These were Baltasound in the north and Dunrossness in

TABLE 1

Constitution of population samples of Amathes glareosa (*from north Shetland to south*)

Area	Numbers caught			*Typica*	
	Typica	*Edda*	Total *	Phenotype	Gene
Unst					
1. Muckle Heog (Baltasound)	292	10359	10651	2·7	16·4
Fetlar					
2. Tresta	34	162	196	17·3	41·6
Yell					
3. Gutcher	38	15	54	70·4	83·9
4. West Sandwick	139	224	364	38·2	61·8
5. Burravoe	23	29	54	42·6	65·3
North Mainland					
6. North Roe	29	47	77	37·7	61·4
7. Hillswick	21	66	87	24·1	49·1
8. Mossbank	58	61	119	48·7	69·8
9. Voe	63	90	153	41·2	64·1
10. Sandness	119	95	214	55·6	74·6
11. Aith	61	74	139	43·9	66·3
12. Reawick	39	57	96	40·6	63·7
13. Kergord	14	9	23	60·9	78·0
14. South Nesting	22	34	58	37·9	61·6
Tingwall Valley: North Border					
15. Catwalls (Gott)	102	33	135	75·6	86·9
16. Scalloway	38	9	47	80·9	89·9
Tingwall Valley: South Border					
17. Braewick (Dales Voe)	27	4	31	87·1	93·3
18. Burn of Dale (Dales Voe)	121	33	154	78·6	88·7
Dunrossness					
19. Cunningsburgh	96	3	99	97·0	98·5
20. Boddam	4364	85	4449	98·1	99·0
21. Levenwick	173	2	175	98·9	99·4
22. Scousburgh	280	10	290	96·6	98·3

* This includes 11 specimens which were classified as intermediate, and may represent another genotype.

the south. Between these sites, samples were taken at a further 17 places. Trapping was continued in each locality until the sample totalled 50 or more or the exigencies of the British tourist season compelled removal. This means that in some places the sample was caught over a period of several days, often from more than one trapping site. The choice of site was usually restricted by the availability of the electricity supply, but in no instance were the sampling points in any one locality more than two miles apart. Successive samples from the same place taken at a later date showed slight but

consistent increases in the proportion of *f. typica* (Kettlewell, 1961*b*), which undoubtedly biased the results to a small extent from different places. It does not, however, affect the over-all interpretation.

3. RESULTS: DISTRIBUTION OF GENE FREQUENCIES

The constitution of the population samples from different places enables the proportion of both forms and the frequencies of the *edda* and *typica* genes to be calculated, assuming the former to be dominant (table 1). It is clear from the table that there are three general groupings: firstly on the two northernmost islands of Unst and Fetlar *f. edda* predominates; secondly on Yell and north Mainland the two forms of the moth are in approximate equality, ranging from about 40 to 60 per cent.; and thirdly, south of a line running just north of the two main towns, Lerwick and Scalloway, *f. edda* becomes rare. The main exception to this general picture is provided by the highly anomalous sample taken in Gutcher, in the northeast of Yell. There is at present no explanation for this population. The sample was taken on two successive nights, and on both the proportion of *f. edda* : *f. typica* was exactly the same. This site obviously should be re-investigated, but meanwhile it remains an exceptional locality.

Another place where a high frequency of *f. edda* is at first sight unexpected is Hillswick. However, the high proportion of *f. edda* found here fits into a trend such that the frequency of *f. edda* increases towards the sea, and Hillswick is on a promontory. The opposite trend is exemplified by the figures for Kergord. Kergord is a watershed on the central hills of Mainland, and is in fact as far from the sea as it is possible to get in Shetland. Despite the fact that Kergord is only 5 miles over the hills from Aith, which is on a sea loch, the frequency of *f. edda* is over 10 per cent. less than at Aith. Previously collectors have reported that *f. typica* was commoner on the top of hills in Unst. We ourselves have no data on this point, but it is possible that at slightly higher altitudes, where on many evenings the hills are enveloped in clouds, visual predation would not take place at the same degree of intensity as in the clear atmosphere nearer sea level.

By making a series of approximate measurements of the distances of places apart, and ignoring minor effects, such as the distance from the sea, it is possible to plot a curve of gene frequency along the land distance in Shetland (fig. 2). Measurements were made of the shortest distances overland from one sampling centre to another. This graph shows both the gene frequency and the phenotype frequency in different areas.

The incidence of *f. edda* remains high over the whole of the northern half of Shetland, falling but gradually (40 per cent. in 45 miles) in a southerly direction. In the south Mainland where its frequency is low, the rate of change is even less (2 per cent. in 12 miles), being only 3 per cent. as far north as Cunningsburgh. It will be seen from fig. 2, however, that a different state of affairs exists in the intervening region.

Here the phenotype frequency of *f. edda* drops 35 per cent. in about 8 miles, although the rate of change of gene frequency in the same area is proportionately less. Now there are two possible explanations of a situation such as this: either the average conditions in the north favour one form and in the south the other and between them there exists free gene flow; or there is an ecological gradient divided by some physical barrier which limits mixing between the two forms. It is possible that in the present case both factors contribute. It is certainly correct to interpret this central area as a boundary region. We were able to detect certain minor ecological differences between the north and the south of this boundary, and these are discussed later. However, there is a physical feature running through the middle of this area which might act as a barrier. This is the Tingwall Valley

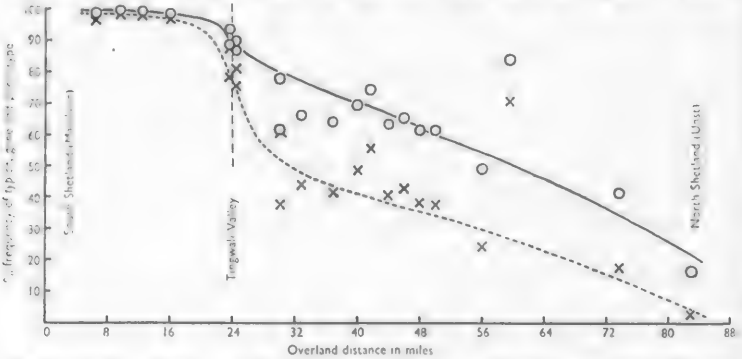

FIG. 2.—Decline in frequency of wild type *Amathes glareosa* and replacement by *f. edda* with distance northwards in Shetland. Phenotype frequencies are represented by X, gene frequencies by O. The dotted line shows the cline in phenotype frequency, the continuous line that in gene frequency.

which runs from southwest to northeast across the island, and varies from one to two miles in width. It consists of fertile farm land quite unsuitable for the requirements of *A. glareosa*. Samples taken on the north side of this valley showed violent fluctuations in frequency from day to day (Kettlewell, 1961*b*) and it can be said with a fair degree of confidence that this feature is likely to be a partial barrier and contribute to the boundary conditions. The significance of this is discussed in the following paper.

4. DISCUSSION

In the discussion that follows it will be assumed that the dimorphism in *A. glareosa* is in a balanced state. Although it is theoretically possible that one of the forms is in the process of spreading, it is unlikely. Evidence on this point would necessitate extensive sampling over a number of years. It can be stated that in 1959 and 1960* the proportions of the two phenotypes were the same at Dunrossness, Hillswick

* And again in 1961.

and Baltasound, within the limits of sampling error. However, the environment in Shetland must have remained substantially the same since the last Ice Age, and it is *a priori* improbable that an advantageous mutation has only recently spread, the more so because of the existence of the same polymorphism in both Fair Isle and the Orkneys. It seems reasonable to assume that the observed composition of the species is stable.

A cline may be the result of a phenotype being at an advantage in one part of a continuous range of a species, while in another it is at a disadvantage. The extremes of the cline in *A. glareosa* are Baltasound (*f. edda* 97 per cent.) and Dunrossness (*f. edda* 2 per cent.). It is between these two places, where neither form is at a great advantage and both forms co-exist, that is our concern here.

The form of any cline is determined by the interplay of two factors:

1. Random migration serving to keep the populations mixed.
2. Selection tending to decrease the incidence of one or the other form.

An analysis of any specific cline must take account of both these agents. It is unlikely that *A. glareosa* actively migrates very far, but in the high wind conditions of Shetland, it may well be involuntarily blown some distance on occasions. The effect of natural selection and habit differences is dealt with more fully in the following paper (Kettlewell, 1961*b*).

The mathematical theory of a cline has been developed by Fisher (1950) and Haldane (1948). Fisher considers a situation involving no dominance, which is of no concern here. Haldane takes the relevant case in which a dimorphism is determined by an autosomal dominant gene and its allelomorph. He analyses the situation when the range of the species (which he assumes to be plane and infinite) is sharply divided into two portions, such that the recessive homozygote has a fitness $1+K$ times that of the other genotypes on one side of the boundary, and $1-k$ on the other. Haldane makes a number of reasonable assumptions in order to carry out this analysis: equal density of the species throughout the area; a single annual generation; random migration, although not necessarily equal in all directions; selection occurring at the point of breeding rather than birth; random mating ; and a population in equilibrium. The essence of the calculation is that the rate of change of gene frequency is the resultant of migration, which will tend to decrease the change, and selection acting differentially on the two phenotypes, which will tend to increase it. For an equilibrium situation these two forces must be equal, and hence Haldane is able to derive an expression for the relation between the intensity of selection, the mean distance migrated per generation, and the slope of the cline. Haldane's calculation is in terms of the relative advantages (= fitness in his terminology) of the

two phenotypes, and the random dispersal distance (m). The assumptions used in making this model are necessarily simplified and formalised, but they seem to be sufficiently realistic to enable us to test whether our data conform to Haldane's theory. It should be emphasised that the assumptions of both Haldane in making the theory and ourselves in presenting the data in the form of fig. 2 (particularly sampling errors, and the difficulty of resolving the distance of places apart to a single dimension), are such that any correspondence between the two must inevitably be approximate. Further, reservations must be entered about two of the assumptions used by Haldane:

1. *A. glareosa* was common wherever we sampled on its correct habitat (heather). On areas away from the heather it was relatively uncommon; on the serpentine of Unst it was very common. Hence it is not quite correct to assume that " the density is equal throughout the area ".
2. Our assumption of a " sharp division of the area of the species by a straight boundary " is based upon *a posteriori* evidence. There can be no doubt about the existence of a barrier in the central Mainland of Shetland, but we never anticipated this until we had sampled the populations at a number of places.

Bearing these two reservations in mind, it is possible to test whether our data conform to Haldane's theory. In order to do this we must assume a value for m and then we can test the goodness of fit of Haldane's model to our figures.

Haldane states a relationship between the distance where the frequency of the recessive gene (y) is 25 per cent. and 75 per cent. (the interquartile range) and the fitness of the phenotypes. In the observed data the interquartile range is about 48 miles and hence

$$k = \frac{1{\cdot}27\ m}{48}$$

(where 1·27 is Haldane's constant when both quartiles are on the same side of the barrier). Now we have as yet no accurate estimate of m, but if we take it as 0·25 miles as the mean for both phenotypes then

$$k = 0{\cdot}00004.$$

Choosing a value of K which puts y at 90 per cent. at the barrier (see fig. 2), we can now test the observed data against Haldane's model. The goodness of fit was calculated only for that portion of the species' range north of the Tingwall Valley since the relatively small change in gene frequency to the south means that many models would not depart significantly from the observed values. Then, omitting the figures for Gutcher for obvious reasons, and those for Baltasound for the reason which appears below, we obtain $\chi^2_{(13)} = 24{\cdot}16$ ($\chi^2_{(13)}$ at the 5 per cent. probability level = 22·36). Although the fit of the data

to Haldane's model is not perfect, this seems a reasonable result in the light of the many approximations discussed above. Hence it seems legitimate to assume that the observed cline could be accounted for by making the same assumptions as Haldane and choosing a random migration distance of 0·25 miles. By choosing larger values of m, it would be necessary to allow a greater intensity of selection to hold the observed cline in existence, but whatever values we choose, it still appears that there is little over-all differential between the two forms. For example, if

$$m = 1 \text{ mile}, k = 0{\cdot}0007, K = 0{\cdot}00063$$
$$m = 5 \text{ miles}, k = 0{\cdot}0175, K = 0{\cdot}0158.$$

This does not negate the fact that heavy selective visual predation may take place, but it does suggest that any such visual advantage is counter-balanced by an equal disadvantage at some period of the life history.

Release-recapture experiments which enable independent estimates (k' and K') to be made of these advantages are described in the following paper (Kettlewell, 1961*b*). It will be seen that *f. edda* was at approximately $7 \pm 6{\cdot}5$ per cent. advantage at Baltasound, but that neither form had an advantage over the other in Dunrossness. For the reasons which are set out, these estimates do not provide a good measure of the selective advantages of the two forms. However, they are in reasonable accord with the estimates of k and K obtained from the frequency data. It would be wrong to make any quantitative deductions from these figures, but three points can be made:

1. There is good cause for believing that the environment of *A. glareosa* on Muckle Heog (Baltasound) is different from that of the rest of Shetland, with the possible exception of Fetlar (Kettlewell, 1961*b*).
2. The calculated value of k is based upon the whole range of the species north of the Tingwall Valley, but the observed value of y at Baltasound is significantly lower than the one expected if Haldane's model can be applied exactly.
3. Both K and K' are small.

The tentative conclusion to be drawn from this is that the conditions for the moth in north Shetland may be such as to alter the relative advantages of the two forms, but over the rest of Shetland there is very little over-all difference between them. However, since the selective differential is so small, there may well be other factors that materially affect the level and stability of the cline. Indeed there must be some factor other than those already discussed to account for the reversal of the differential survival rates to the north and the south of the Tingwall Valley. It is therefore in place to consider other factors that may be involved in the cline.

1. It has been shown that *f. edda* from Unst have behaviour differences as well as colour differences from south Shetland *f. typica* (Kettlewell, 1961*b*). Because of the former it could be that *f. typica* is at a reproductive advantage in regions of certain gene frequencies whilst being at a cryptic disadvantage in the same area.

2. De Ruiter (1952) has shown that when a bird has experienced a particular cryptic insect as an article of food it subsequently tends to search for others of the same model. Cain and Sheppard (1954) have pointed out that if one can conclude from this that the exploitation of one form decreases for a time the chance of finding another, then in a polymorphic species the more abundant form will be proportionately more heavily predated. Hence the uncommon forms would be at an advantage because of their scarcity. If the habits of *f. edda* and *f. typica* are distinct from the predators' point of view, it might be beneficial to the species for both forms to occur together. Such an effect would contribute to the cline, and is not contrary to any of the assumptions involved in making the mathematical model.

3. There is some slight evidence that mating is non-random, and that there is a tendency for opposite phenotypes to pair. Sheppard (1953) has described such an effect in the moth *Panaxia dominula* L. and he pointed out that this could be at least one of the factors tending to ensure stability in a polymorphism.

4. A balanced polymorphism results if the heterozygotes are at an advantage over both homozygotes. We have no evidence about the presence or absence of any heterosis.

This discussion of the cline has assumed that Haldane's model can be applied. Haldane only considers in detail the case of a species divided by a "sharp boundary". Professor Haldane has pointed out to us that in his theory he assumes only a "boundary", not a barrier to random or other migration. He suggests that the Tingwall Valley may be perhaps as effective as ten miles of heather, and hence the selective intensities may be greater than we have calculated. It is possible that the effects of a gradual change of conditions over the length of Shetland are imposed as well. The fact that *f. edda* also occurs (but at a low frequency) both on Fair Isle to the southwest and Orkney to the south, is in favour of this. This much can be said, however, we have observed no obvious major ecological differences between north and south. We have, however, noted minor ones. Over much of the northern half (with the exception of the small outcrops of serpentine) large areas of peat-hag are evident, the degree of erosion depending on the number of sheep grazing, the activity of the local inhabitants in peat digging and the degree of exposure to winds. In south Shetland there is less peat-hag and more rock, and there is also more cultivation. Also at the extreme south there are considerable areas of sand dunes and we found the species here also.

A further and possible relevant observation is that the average

heather depths which we tested in the extreme north and south were different. Thus on Unst (serpentine) the deepest heather over two stretches of 200 yards each, sampled at points taken at 10 yard intervals, was 1·6 inches. On each occasion the deepest heather available within one yard of the point was chosen, this probably being the greatest distance *A. glareosa* would walk after alighting before taking up a resting position. Using the same methods in the south the mean depth was 3·8 inches. Furthermore, over much of north Mainland and Yell we found that the surfaces of exposed peat, uncovered by vegetation, were much more extensive than in the south.

F. edda is highly cryptic resting on peat, whilst *f. typica* is not, unless covered with vegetation. These two factors—depth of vegetation and bare peat surfaces—may in fact play an important role in determining a differential cryptic efficiency between the two forms.

In the absence of more definite and obvious ecological differences between the north and south Shetland, it seems best therefore to accept, with reservations, the application of Haldane's model for the present and the implications that follow from it.

It is worth while to mention the example used by Haldane (1948) to illustrate his theory of a cline. He considered a cline of de-pigmentation in the deer-mouse, *Peromyscus polionotus*, described by Sumner (1932), and he found low values of K and k. From this he concluded that the mutant variety was " probably formed under the action of much more intense selection on the beaches " (the extreme of its range). This is interesting because we have to postulate much the same effect for the cline in *f. edda*, but here we have obtained the additional information that there is more intense selection operating at the extreme of its range in Unst. This is discussed more fully in the following paper.

In conclusion it is pertinent to point out specifically that the method of analysis of the polymorphism described in this paper is powerless to indicate the actual forces of selection involved. These may in fact be very strong and work in opposing directions: the frequency data reveal only the average conditions of the species throughout its life and the mean selective coefficients which contribute to the polymorphism.

5. SUMMARY

1. The moth *Amathes glareosa* Esp. exists in a dimorphic state in the Shetland Islands, the typical form occupying 98 per cent. of the population at the south of the group and being progressively replaced by a distinct melanic form, *edda*, in a northerly direction so that 97 per cent. of the population is *f. edda* at the north of the group.

2. Population samples were taken at over twenty places throughout the islands, and the gene frequencies from north to south plotted. A frequency map was constructed from over 20,000 moths caught.

3. It was found that a model for a cline suggested by J. B. S. Haldane appeared to fit the data with certain exceptions. Hence it

was possible to calculate the selective forces necessary to maintain the cline in being, assuming a random dispersal distance of the moths.

4. The selection coefficients involved in the cline are small although the biological pressures are high, but these do not differ significantly from independent estimates of differential selection between the two forms of the moth obtained by release-recapture experiments. Hence it is possible that the cline is maintained entirely by differential predator selection, although it is unlikely that this is so.

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SELECTION EXPERIMENTS ON MELANISM IN *AMATHES GLAREOSA* ESP. (LEPIDOPTERA)

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INTRODUCTION

THE Noctuid moth *Amathes glareosa* Esp. is widely distributed throughout the Shetland Isles (Kettlewell and Berry, 1961). It has been established that at the north end of the islands 97 per cent. of the population are of the melanic form *edda*. By contrast on south Shetland Mainland, less than 60 miles away, the population is 98 per cent. of the light form. In the intervening region there is a steady frequency cline which is interrupted by a rather rapid change near the Tingwall Valley, which appears to act as a partial barrier. Fuller details of the two contrasting populations to the north and to the south must be recorded here.

1. THE STRUCTURES OF THE TWO POPULATIONS

(i) *Unst, North Shetland*

In 1959 a total of 2539 *A. glareosa* were examined by C. J. Cadbury. Of these 77 were *f. typica* (3·03 per cent.). This sample was smaller than that taken in 1960, because of an insufficient number of mercury vapour traps on which we depend for sampling. The total captures in each of these years, therefore, bears no direct relationship to the abundance of the species.

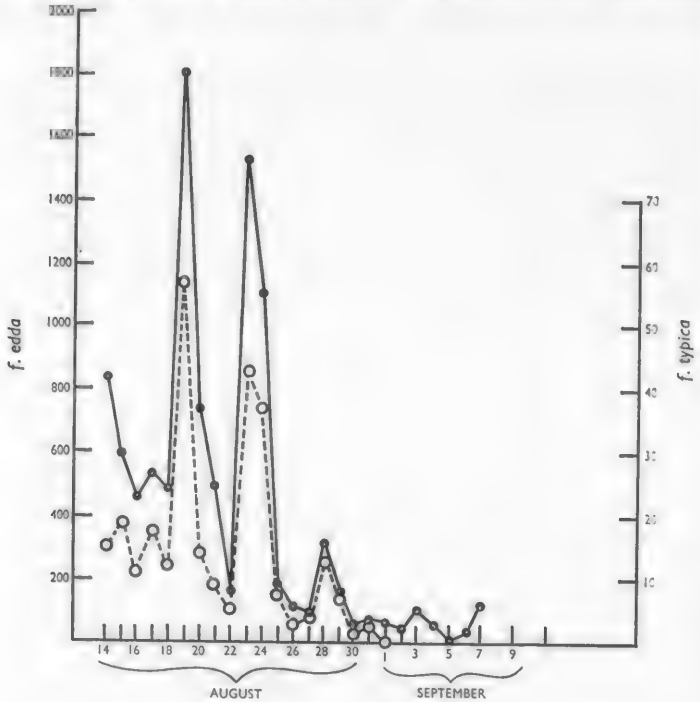
In 1960 10,650 *A. glareosa* were caught of which 294 were *f. typica* (2·76 per cent.) (text-fig. 1). In the first week 90 per cent. of the *A. glareosa* were males but at the end of the season a higher proportion were females. Table 1 shows nightly catches for the season along with the percentage of *f. typica*. There is some evidence that the frequency of *f. typica* in the north increases throughout the hatching period. This will be discussed later.

(ii) *Dunrossness, South Mainland*

The structure of the population is very different here. Of 5013 *A. glareosa* which were captured in 1960, 4913 were *f. typica* (98 per cent.). There was no evidence that *f. edda* appeared earlier than *f. typica* in these figures, nor would it be expected if predation is relaxed in south Shetland, since there would then be no selection in favour of the later emergence of either form.

This situation of two contrasting populations appeared to offer exceptional opportunities for analysis by experimentation in the field. With the help of a team already trained in the special techniques, I decided to undertake extensive mark-release-recapture experiments both in north and south Shetland, the two to run concurrently. By

choosing the opposite ends of the Shetland Isles as centres of our experimental releases where the frequencies of the two forms showed their greatest contrasts, I had hoped to be able to evaluate the results of selective predation in the same way as had been possible in industrial melanism. The method employed was to catch and mark large numbers of each form at both locations. A proportion of the commoner



TEXT-FIG. 1.—Wild captures of the two forms of *Amathes glareosa* taken in mercury vapour traps, Unst 1960 (continuous line *f. edda*).

of the two forms was then transported as rapidly as possible to the opposite end of the islands. In view of the fact that the distances involved were small, it has been assumed that each phenotype, *regardless of origin*, was identical with another of the same form in regard to its behaviour. Further work will confirm or deny this.

Unfortunately in carrying out a design of experiment as complicated as this we met with considerable difficulty. There are two sounds to be negotiated, firstly between the islands of Unst and Yell, and secondly between Yell and north Shetland Mainland. Nevertheless, with the collaboration of the Shetlanders, a parcel of live insects was sent each day from north to south and another from south to north. This demanded the daily use of four separate omnibus services and two

ferries in each direction. The specimens were contained in pill boxes, 2 in a box, and it is fair to the Shetlanders to put on record that on no single occasion were any of the 44 parcels either damaged or lost. All the moths arrived in excellent condition and by this means we had a plentiful supply of the rarer of the two forms at each of the opposing centres of mark-release. Because of the journey, however, 36 hours elapsed between the catching of the moths and their subsequent release. We were punctillious throughout therefore to ensure that the locally caught specimens were similarly incarcerated for 36 hours, 2 to each pill box. This shuttle service continued daily (except Sunday) from 14th August till 5th September.

2. MARKING AND RELEASING TECHNIQUES

Marking techniques were modelled on those devised by Dowdeswell, Fisher and Ford (1940). I have previously used modifications of these on a number of Lepidopteran species with satisfactory results. *A. glareosa*, however, presented particular difficulties. A small scuttling Noctuid moth, whose hindwings project almost as far as the forewings, and which at the same time are plicated like a fan, was found to be very different from those species with which I have previously worked. The lethargy of *Biston betularia*, the surface area of *Lasiocampa quercus*, and the convenient white spotting of *Panaxia dominula* were all absent. Nevertheless we eventually developed a technique for successful marking without the use of an anæsthetic which at one time we thought would be necessary. This depended on a method of handling the moth with forceps in such a way that the wings were dorsi-flexed and ensuring that they were not stuck together after applying the paint spot.

Using a combination of the undersides of four wings, and five colours, we were able to identify twenty individual markings, and we found this adequate for the life-span of the species. In 1959 a small pilot experiment had been undertaken on Unst (Site 1), but because of an inadequate knowledge of the natural history of the moth, an insufficient supply of *f. typica* from south Shetland was available. In 1960 we commenced mark-release experiments on the same releasing site (Site 1), but this was abandoned after three days because, unlike 1959, there was no evidence of birds feeding in the area. The new site chosen (Site 2) was separated from the first by a half-mile stretch of sea. It was situated on the side of the hill of Muckle Heog to the north of Baltasound. Geologically this area consists of serpentine rock and as such is a specialised biotope maintaining many rare species of small plants (Spence, 1957). Due to the toxic nature of the soil the vegetation is sparse and the peat-coloured soil is shallow, though no depth of peat is present.

On our first visit in 1959 we arrived on 1st August. Subsequently we found *A. glareosa* extremely common here. Furthermore, birds were active on the ground not only by day but throughout most of the night. After mid-August, however, the hours of darkness rapidly increase

at this latitude, so that in 1960 when we arrived two weeks later we observed no twilight activity by birds whatever. Nor did we record any at full moon which was on 5th September. Diurnal feeding, however, was very much in evidence on Site 2.

TABLE 1

Wild population captures for Amathes glareosa *and its melanic form* edda, *Unst, N. Shetland, 1960*

	Date	Phenotype		Total	Per cent. *typica*
		Typica	*Edda*		
Site 1	13.8.60	0	3	3	...
	14.8.60	15	835	850	1·765
	15.8.60	19	611	630	3.016
	16.8.60	19	465	476	3·311
	17.8.60	18	629	647	2·782
	18.8.60	12	485	497	2·415
Site 2	19.8.60	54	1,792	1,846	2.925
	20.8.60	14	743	757	1.849
	21.8.60	9	484	493	1·826
	22.8.60	6	166	172	3·488
	23.8.60	43	1,530	1,573	2·734
	24.8.60	37	1,111	1,148	3·223
	25.8.60	7	225	232	3·017
	26.8.60	3	110	113	2·655
	27.8.60	4	88	92	4·348
	28.8.60	13	320	333	3·904
	29.8.60	7	160	167	4·192
	30.8.60	3	58	61	4·918
	31.8.60	5	68	73	6·849
	1.9.60	0	61	61	...
	2.9.60	1	41	42	2·381
	3.9.60	4	99	103	3·883
	4.9.60	2 −14	54 −473	56 −487	3·571 −2·875
	5.9.60	0	5	5	...
	6.9.60	4	30	34	11·765
	7.9.60	2	121	123	1·626
	8.9.60	1	62	63	1·587
		294	10,356	10,650	2·761

Total captures = 10,650
Total *typica* = 294
Total *edda* = 10,356
Average percentage of *f. typica* = 2·761

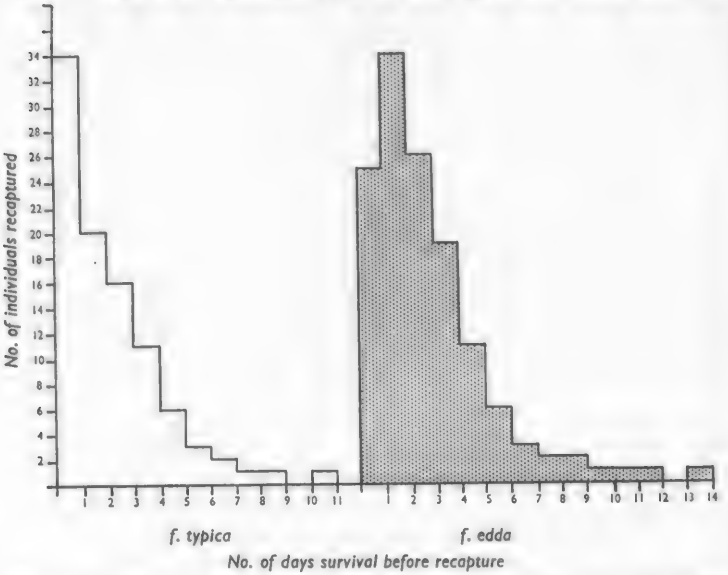
This new releasing ground was approximately one-quarter square mile in extent and from 18th August till 5th September large numbers of *A. glareosa* were released daily, each form being as near to equality as possible. Because of the eccentricities of the omnibus services the release was not practicable before 4 p.m. The time available for predation on the first day was therefore limited to a few hours. After release the behaviour of both forms followed a definite pattern. Both fed for a short period on heather flowers and then took up their

1960 Site 1	Release		Day 1		2		3		4		5		6		7		8		9		10		11		12		13		14		Total recaptures		Total both forms NOT recaptured
	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T	E	
14.8 . .	46	3	0	0	0	0	3	0																							3	0	46
15.8 . .	95	295	2	0	1	4	2	5																							5	9	376
16.8 . .	106	199	1	2																											1	2	302
Total . .	247	497																															724
Site 2																																	
18.8 . .	95	182	10	4	3	6	2	0	0	1	3	0	1	2	0	0	0	0	0	0	0	0									19	13	245
19.8 . .	223	229	5	6	1	3	1	0	4	4	2	5	0	0	0	0	0	0	0	0											13	18	421
20.8 . .	179	230	0	0	0	1	2	7	4	3	0	0	0	1	2	0	0	1	0	0	0	0									8	13	388
21.8 . .	193	245	0	0	6	8	5	9	0	2	0	2	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	12	23	403
22.8 . .	149	150	13	7	3	6	1	0	0	2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	17	18	264
23.8 . .	7	9	0	0	0	0	0	0	0	0	1	0	0	0																	1	2	13
24.8 . .	223	213	3	2	1	1	1	1	0	3	0	0	0	0	0	2	0	1	0	0	0	1	0	1	0	0	0	0	0	0	5	12	419
25.8 . .	155	243	0	0	1	2	1	6	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	4	11	383
26.8 . .	70	81	1	0	0	3	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0							2	5	144
27.8 . .	18	36	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0									1	2	51
28.8 . .	93	86	0	2	0	0	0	0	1	0	0	0	0	0																	1	2	176
29.8 . .	176	166	0	1	3	4	1	1	0	0	0	0	0	0	0	0	0	0	0	1											4	7	331
30.8 . .	148	102	0	0	0	0	1	2	1	1	0	0	0	0																	2	3	245
31.8 . .	74	35	0	0	0	0	1	0	0	0	0	0																			1	0	108
1.9 . .	81	44	0	0	0	0	0	0	0	0	0	0	0	1																	0	1	124
2.9 . .	51	32	1	0	0	0	0	0	1	0	0	1	1	0																	3	1	79
3.9 . .	52	40	0	0	0	0	0	0	0	1																					0	1	91
4.9 . .	52	83	0	0	1	0	0	0																							1	10	134
5.9 . .	45	54	0	0	1	0	0	0																							1	0	98
Totals . .	2084	2260	34	25	20	34	16	26	11	19	6	11	3	6	2	3	1	2	1	2	0	1	1	1	0	1	0	0	0	1	95	132	4117
	4344		1		2		3		4		5		6		7		8		9		10		11		12		13		14		4344		
			Day																														

Typica days = 256 *Edda* days=440

characteristic resting positions on the ground and from then on rarely moved till dark.

Around this releasing ground and at distances of approximately 200 to 500 yards we had three concealed 125-watt mercury vapour traps. These were turned on each evening at early dusk and off at dawn. The highly successful type of trap we have now developed



TEXT-FIG. 2.—Histograms of marked recaptures of both forms of *Amathes glareosa* showing the number of days' survival in the wild population (from 19 separate releases), Unst 1960.

captures, we believe, random samples of the insects which are in flight. A similar design of sampling was employed at each of the releasing sites.

3. RELEASE DATA

We are here concerned with the recapture results of three release experiments undertaken in this way in 1960; two on Unst on north Shetland and one in the south Mainland. On Unst 90 per cent. of the *f. typica* released came from south Shetland: in Dunrossness 92 per cent. of *f. edda* were imported from Unst.

Unst Site 1: Total release 744 *A. glareosa* (*edda* 497; *typica* 247). This site was abandoned after three days for the reasons given above.

Unst Site 2: Total release 4344 *A. glareosa* (*edda* 2260; *typica* 2084).

Dunrossness, south Shetland: Total release 2144 *A. glareosa* (*edda* 961; *typica* 1183).

Tables 2 and 3 show the distribution of subsequent recaptures and text-fig. 2 depicts the histograms of recaptures of each form from Unst

TABLE 3

Release-recapture figures of both forms of Amathes glareosa, *South Mainland, 1960*

Release date	Release		No. of days between release and recapture														Recaptures Total		Unrecaptured moths		
			1		2		3		4	5	6	7	8	9	10	11					
			T	E	T	E	T	E	T E	T E	T E	T E	T E	T E	T E	T E	T	E	T	E	Total
20.8.60	60	96	7	1	0	1	2	1	2 1	...	...	...	...	...	...	...	11	4	51	92	143
21.8.60	170	1	1	0	2	0	3	0	3 0	...	...	...	...	...	...	...	9	0	161	1	162
22.8.60	159	167	1	2	3	4	0	1	0 0	0 1	1 1	0 0	0 0	0 1	0 0	0 1	5	11	154	156	210
23.8.60	13	1	0	0	1	0	0	0	0 0	1 0	...	...	...	...	...	...	2	0	11	1	12
24.8.60	70	105	0	0	1	0	0	1	1 0	0 1	0 0	0 1	...	...	...	...	2	3	68	102	170
25.8.60	172	156	1	0	1	0	0	3	0 0	0 0	0 1	...	...	...	...	...	2	4	170	152	322
26.8.60	72	71	0	0	1	2	0	0	0 0	0 1	...	...	...	...	...	...	1	3	71	68	139
27.8.60	5	34	1	0	0	1	0	1	.....	...	...	...	...	...	...	...	1	2	4	32	36
28.8.60	1	3	...		...		...		...	...	...	...	...	...	...	...	0	0	1	3	4
29.8.60	97	154	0	0	2	4	2	4	0 0	1 1	1 3	0 0	0 0	1 4	...	...	7	16	90	138	228
30.8.60	130	52	0	0	2	0	2	1	2 2								6	3	124	49	173
31.8.60	46	25	1	0													1	0	45	25	70
1.9.60	39	45	0	0	0	1	0	0	0 0	0 0	2 0						2	1	37	44	81
2.9.60	79	40	1	0	0	0	0	0	0 0	2 2							3	2	76	38	114
3.9.60	19	3															0	0	19	3	22
4.9.60	48	5															0	0	48	5	53
5.9.60	1	3	0	0	0	1											0	1	1	2	3
	1183	961	13	3	13	14	9	12	8 3	4 6	4 5	0 1	0 0	1 5	0 0	0 1	52	50			
			1		2		3		4	5	6	7	8	9	10	11					

TABLE 4

Observed and expected recaptures of both forms of Amathes glareosa *for day 1, day 2 and for the total period, together with variance*

1960 North End Site 1 Unst, Shetland)	Day 1			Day 2			Total recaptures all days		
	Obs. *edda*	Exp. *edda*	Var.	Obs. *edda*	Exp. *edda*	Var.	Obs. *edda*	Exp. *edda*	Var.
14th August	0	...	...	0	...	...	Site abandoned after 3 days		
15th	0	1·513	0·368	4	3·782	0·912			
16th	2	1·957	0·676	0	...	...			
Total Site 1 .	2	3·470	1·044	4	3·782	0·912			
Site 2									
18th August . . .	4	9·199	3·006	6	5·913	1·969	13	21·025	6·401
19th	6	5·573	2·689	3	2·027	0·993	18	15·706	7·233
20th	0	...	...	1	0·562	0·245	13	11·809	4·915
21st	0	...	...	8	7·831	3·348	23	19·578	7·955
22nd	7	10·033	4·681	6	4·515	2·190	18	17·559	7·751
23rd	2	1·125	0·459	0	...	...	2	1·600	0·640
24th	2	2·443	1·238	1	0·977	0·499	12	8·305	4·092
25th	0	...	...	2	1·832	0·710	11	9·158	3·441
26th	0	0·536	0·249	3	1·609	0·736	5	3·755	1·671
27th	1	1·353	0·436	0	...	...	2	2·000	0·642
28th	2	0·961	0·496	0	...	...	2	1·441	0·740
29th	1	0·485	0·250	4	3·398	1·718	7	5·339	2·667
30th	0	...	...	0	...	...	3	2·040	1·188
31st	0	...	...	0	...	...	0	0·321	0·216
1st September	0	...	...	0	...	...	1	0·352	0·228
2nd	0	0·386	0·237	0	...	...	1	1·542	0·913
3rd	0	...	...	0	...	...	1	0·435	0·246
4th	0	...	...	0	...	...	0	0·615	0·237
5th	0	...	...	0	...	...	0	0·545	0·248
Total Site 2 . .	25	32·074	13·741	34	28·664	12·408	132	123·125	51·426
							Diff. : 8·875±7·1712 (Standard error) 1·24 = P>0·21<0·22 = 7 per cent. diff. in favour of *f. edda*		

releases site 2. A full analysis of the south Shetland experiment is not given because both the release techniques and the site chosen were considered unsatisfactory.

4. ANALYSIS OF RECAPTURE DATA

On comparing the histograms of the two forms for the main release in the north, in which recaptures are recorded according to the number of days' survival of each form in the wild, two main points stand out. Firstly there is a deficiency of *f. edda* recaptures on the first day. On the other hand the histogram for *f. typica* has a distribution according

to expectation. In all release experiments more recaptures are to be anticipated on the day immediately after release than on subsequent days because of depletion by predation, dispersal or death from other causes. The deficiency of first day recaptures of *f. edda* is therefore

TABLE 4—*continued*

1960 South End (Dunrossness, Shetland)	Day 1			Day 2			Total recaptures all days		
	Obs. *edda*	Exp. *edda*	Var.	Obs. *edda*	Exp. *edda*	Var.	Obs. *edda*	Exp. *edda*	Var.
20th August	1	4·861	1·822	1	0·608	0·238	4	9·114	
21st	0	0·006	0·006	0	0·012	0·012	0	0·053	
22nd	2	1·537	0·745	4	3·586	1·717	11	8·196	
23rd	0	...	...	0	0·071	0·066	0	0·143	
24th	0	...	...	0	0·600	0·240	3	3·000	
25th	0	0·476	0·249	0	0·476	0·249	4	2·854	
26th	0	...	...	2	1·490	0·739	3	1·986	
27th	0	0·872	0·112	1	0·872	0·122	2	2·615	
28th	0	...	...	0	...	...	0	...	
29th	0	...	...	4	3·681	1·394	16	14·112	
30th	0	...	...	0	0·571	0·406	3	2·571	
31st	0	0·352	0·228	0	...	...	0	0·352	
1st September	0	...	...	1	0·536	0·249	1	1·607	
2nd	0	0·336	0·223	0	...	...	2	1·681	
3rd	0	...	...	0	...	...	0	...	
4th	0	...	...	0	...	...	0	...	
5th	0	...	...	1	0·750	0·188	1	0·750	
Total South Shetland	3	8·440	3·385	14	13·253	5·610	50	49·034	
Combined totals of 3 experiments	30	43·984	18·170 P = 0·001	52	45·699	18·930 P = 0·15	Therefore no evidence of selective predation in figures		

There is no heterogeneity for day 1 recaptures in the three release experiments, nor for day 2. The results can therefore be combined for each day separately.

For day 1 recaptures P = 0·001
For day 2 recaptures P = 0·15

Therefore there is a highly significant deficiency of *f. edda* recaptures on day 1, compared to *f. typica*.

quite exceptional and was unexpected. If this departure from expectation was significant it could be of importance.

A second point shown by the histograms is that the total *f. edda* recaptures (assuming equality of release) was greater than that of *f. typica*.

5. DISCUSSION 1

A. Evidence of a behavioural difference in the wild

A comparison of the proportions of recaptures of the two forms on day 1 shows that there is a deficiency of *f. edda* on that day and that the results are homogeneous in all three experiments (table 4). If we

consider the results of our main experimental work in the north (Unst site 2), we can calculate the mean life-span of both phenotypes after release, for *f. edda* 3·41 days, for *f. typica* 2·69 days. Assuming these to be constant for each form the expected number of recaptures can be assessed for each day. Hence we can test whether the observed recaptures deviate from the expected. They do not (for *edda* $\chi^2_{(13)} = 12{\cdot}29$; for *typica* $\chi^2_{(11)} = 1{\cdot}79$) except for the *edda* recaptures for the first day after release on which there is a significant deficiency of recaptures ($\chi^2_{(1)} = 4{\cdot}85$; $P = >0{\cdot}02, <0{\cdot}05$).

Another method of considering this is to take the total recaptures of both forms on both day 1 and day 2 and to compare each of these with the number expected along with its variance (tables 4 and 4*a*). This method is based upon a technique developed by C. A. B. Smith

TABLE 4 (*a*)

A comparison of day 1 and day 2 recaptures from three mark-release-recapture experiments, Shetland, 1960 (summary of table 4)

	Day 1	Day 2
Observed *edda* . . .	30	52
Expected *edda* . . .	43·984	45·699
Variance	18·170	18·930
	P = 0·001	P = 0·15

for sibship studies in duodenal ulcers (Clarke *et al.*, 1956). In this we have had to make the assumption that the two forms have equal death rates. This is probably near the truth for the first day after release.

When the recaptures for day 1 and day 2 for all three experiments are considered in this way, the probability of such a deficiency of *f. edda* occurring on the first day by chance is 1 in 1000. An explanation of this is unlikely to be accounted for by selective predation in favour of *f. typica* seeing that this did not occur on subsequent days. Furthermore, because of the late hour of release on the previous day, little, if any, predation could have actually taken place. This immediately suggested that our method of sampling was in error and that the absence of *edda* on day 1 might reflect a habit difference. It seemed likely in fact that *f. edda* (of local Unst origin) took flight less frequently than *f. typica* (from south Shetland) and that this habit difference was reflected in our trap samples which depended on taking random moths on the wing.

It must here be pointed out that flight in the Shetland Isles in common with other small islands, is a hazardous occupation for most insects, particularly if undertaken near the coast. It will be recalled how on certain islands in other parts of the world apterous species have developed because of this. The average wind strength throughout

the year in north Shetland is similar to that at the top of Ben Nevis. Nevertheless even under these conditions there must be compensating advantages for flight such as a capacity for finding a mate, an opportunity for feeding or ovipositing on suitable plants, and an assurance of adequate gene-flow. Maximum land dispersal with minimum loss through drowning must in fact conflict in most island species.

We are able to provide further evidence that a habit difference between south Shetland *f. typica* and northern *f. edda* exists. Equal numbers of both forms (25 of each) were marked and released into an area of approximately 10 square yards at 8 p.m. All the moths took up resting positions after having previously fed. From then till dark I was in a close position to observe them and see that no bird predation took place. The first sign of activity took place at late dusk (9.55 p.m. G.M.T. 18th August). Fifteen *f. typica* were seen to fly before 11 p.m., but only 5 *f. edda*. This could be accounted for because of the greater ease of seeing the light coloured *f. typica* on the wing. However, using a torch a count of 12 *edda* to 3 *typica* ($\chi^2_1 = 4{\cdot}3$) was made at midnight as they fed or rested on the heather. It was apparent that most of the *f. typica* had flown. Furthermore, twenty-four hours later on the following evening 5 marked *edda* were found but no *f. typica* in spite of this form being so much more conspicuous. It is hard to construe these observations in any way other than that under certain conditions *f. edda* takes flight less frequently than *f. typica*, but it must be remembered that the majority of our *f. typica* come from south Shetland.

Independent evidence of a habit difference was obtained by each of us who were responsible for undertaking the daily releases for our main experiments. On being thrown into the air *f. typica* would frequently fly a short distance before settling: *f. edda* was much more lethargic and usually fell to the ground.

Evidence that this habit difference may be associated with the two forms is provided from our sample data from the Tingwall Valley which is situated in the centre of the Mainland barrier zone (Kettlewell and Berry, 1961). In contrast to our usual finding that the frequencies of our samples vary but little from one night to the next, here on the north side of the valley, nightly frequencies fluctuated widely. Heterogeneity by the contingency test is $\chi^2_{(4)} = 13{\cdot}10$. There is some evidence also that on many nights the frequency of *f. typica* on the north side was inversely proportional to that on the south. Though these observations were made at a period when the species was diminishing in numbers and the samples were therefore small, the most reasonable explanation is that the fluctuations were brought about by a differential flight habit between the two forms. The situation at the Tingwall Valley is that the population to the south is largely composed of wild type. To the north, however, the melanic form rapidly becomes more common. Part of the 1961 expedition will be attempting to find out more about the habits of the two forms in this most interesting area. We have, however, already provided

evidence that different behaviour patterns occur within the species in the wild.

This is the first occasion on which a habit difference has been demonstrated in the wild by mark-release methods in a polymorphic insect. Hovanitz (1948), by other methods demonstrated an activity difference in the white mutant female of *Colias* butterflies. It is likely that behavioural differences of this kind will be found much more commonly in future and that they will be seen to contribute materially to most balanced polymorphisms.

Mechanism and origin of habit difference. A differential flight activity could be brought about by two distinct mechanisms. Either activity could be controlled by genetic factors or if associated with *f. edda* it could be the direct consequence of the physical difference between black and white bodies. If genetic in origin the behavioural difference could be pleiotropic or it could be controlled by one or more modifying genes which have become linked to the gene responsible for the pigmentation of *edda*. Alternatively it could have arisen independently of either form at the opposing ends of a population subject to intensive selection under high wind conditions on small islands and headlands, and where gene-flow was limited between the north and south ends. It has been pointed out that habit differences are more likely to be found in long-standing balanced polymorphisms than in the comparatively recent one of industrial melanism (Kettlewell, 1961).

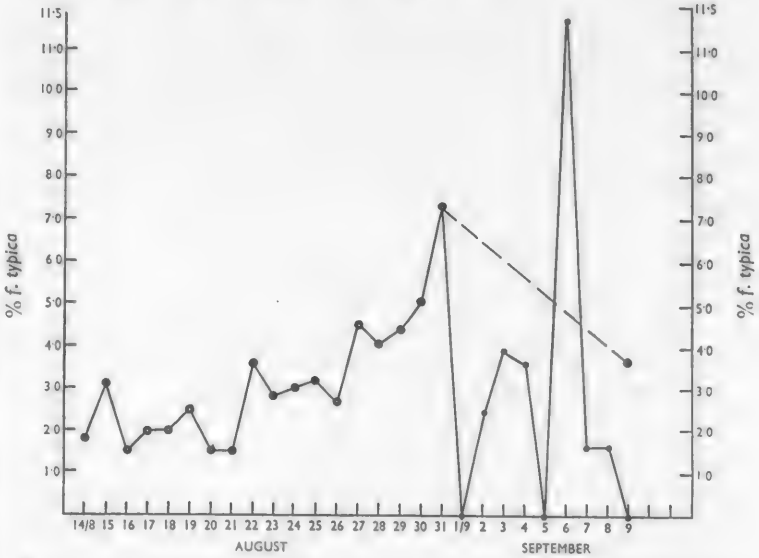
If decreased flight activity is associated with the melanic *f. edda*, a simple physical phenomenon could account for this as a direct consequence of black colouration. It is necessary for Noctuid moths to vibrate their wings for a considerable period of time before taking flight and this is in order to increase their internal body temperature. The temperature on Unst at night is fairly constant in the month of August and seldom rises above 55°F. It is possible that on colder nights the heat lost from the black surfaces of *f. edda* is more rapid than the heat produced and that this does not take place in the same way with the light coloured *f. typica*. We intend to check this by using thermo-coupling in the near future.

B. Evidence of a different time of emergence

If the frequencies of *f. typica* in the daily samples of the wild population on Unst shown in table 1 are graphed (text-fig. 3) it will be seen that there is a steady increase of this form throughout the period of hatching.

After the end of August it is likely that the majority of *A. glareosa* pupæ have emerged, the population density is in a decline, the samples get smaller and *f. typica* will be disappearing from the population, due to selective predation, more rapidly than *f. edda*. These facts probably account for the drop in the *typica* frequency after 31st August. There are proportionately more *f. typica* in the population in the

week after peak emergence than before. Williamson (1956) has observed on Fair Isle that *f. edda* (where the phenotype frequency is 30 per cent.) appeared in advance of *f. typica*. Three *edda* were caught on 4th August 1955, but no *f. typica* were taken until one week



TEXT-FIG. 3.—Graph showing daily percentage of *f. typica* in the wild population on Unst 1960.

later. If the wild type occurs more frequently in samples collected later in the hatching period there are three possible explanations:

1. That the figures directly reflect a deficiency of *f. typica* in the earlier part of the season as the result of selective crepuscular predation which is possible at this time, but not later. Because of the longer hours of darkness which come on rapidly towards the end of August, crepuscular predation may be relaxed. We have been able to show that under conditions of twilight *f. typica* is at a cryptic disadvantage. The average maximum distance that three of us could see *f. typica* in the twilight or full moon, as it fed on heather flowers, was 12 feet: for *f. edda* it was 2 feet.
2. That because of the increased number of hours of darkness late in the season, *f. typica* which may fly more freely than *f. edda*, has greater opportunities than *f. edda* of coming to light traps.
3. That *f. typica* emerges later in the hatching period and that this is controlled genetically and adjusted by selective pressure. Natural selection would favour those *typica* which hatched later and hence were protected by darkness.

In regard to explanations 1 and 2 our recapture data do not show that a larger proportion of marked *f. typica* disappeared from our releases in the first half of the experiment than in the second. Nor do they provide evidence that marked *f. typica* were recaptured at light more freely later in the season. In fact we recaptured 6·25 per cent. of marked *typica* during the first nine days and 1·89 per cent. of our releases during the last ten. It is likely, therefore, that in Unst this seasonal increase in the frequency of *typica* is also a habit difference and that the majority of *f. typica* pupæ hatch at a somewhat later date than those of *f. edda*. Selection for this may have taken place only on those islands which are subjected to intensive bird predation. It is therefore more likely to be observed in populations maintaining a moderately high frequency of *f. edda*.

6. DISCUSSION 2

A. Evidence of predation and of differential survival in the wild

Direct observation on predation. As early as 1876 Buchanan White (1876) recorded that gulls and other birds scouted moths on the wing in twilight in Shetland. More specifically referring to *Hepialus humuli* (figured Kettlewell, 1961*a*), he states that the white typical forms must be very conspicuous in flight and that the darker forms would be at an advantage under the prevailing conditions. Similar observations have been made by Griffith (1929) who recorded gulls hawking male *H. humuli* for twenty to thirty minutes at dusk. Venables and Venables (1955) also comment on how the gulls appear quite suddenly as soon as the *H. humuli* flight commences and leave after half an hour when it is over. In 1960 we observed the common gull taking on the wing both forms of *Arenostola pygmina* Haw. which flies in the late afternoon. In 1959 in early August we saw many species of birds feeding on the ground both by day and during the long twilights. We had anticipated that *A. glareosa* would be taken on the wing in the same way as *H. humuli* but saw no evidence of this. To test this we impaled equal numbers of both forms alive on small fish hooks and these were allowed to fly at dusk suspended from very fine nylon stretched out over distances of approximately 100 yards. C. J. Cadbury observed that neither form was predated. He also noted that both forms of *A. glareosa* had whitish hindwings and that they exposed them in flight. This suggested that it was unlikely that aerial predation comparable to that which is known to take place in *H. humuli* was the main selective force at work. If predation in flight was excluded there remained two other vulnerable periods in the course of a day. The moth could be eaten (i) whilst at rest on the ground by day or (ii) whilst feeding on heather flowers or ovipositing in the twilight.

(i) *Evidence of* A. glareosa *predation by day.* We constantly saw birds feeding by day on the hillsides through binoculars. The most frequent

species were the common gull *Larus canus* L., the wheatear *Œnanthe œnanthe* L., the peewit *Vanellus vanellus* L., the golden plover *Charadrius apricarius* L. and the starling *Sturnus vulgaris* L., the last two species frequently being in flocks. The common gull, up to 50 per cent. of whose diet has been shown by Spärck (1950) to consist of insects, was because of its size likely to be a formidable predator. Breeding common gulls are common throughout the north Shetland Isles but probably less so in the south (Venables, personal communication). They are also augmented by migratory flocks from the north in mid-August (Venables and Venables, 1955).

I therefore decided to shoot a minimum number of gulls during daylight. This was limited to five in all, three in August and two subsequently, both of which had not been feeding and had empty stomachs. Of the three, one was killed on our release ground and the two were half a mile from it. Of the latter, gull 1 contained 8 *A. glareosa*, one of which was *f. typica*. Three specimens of *Cerapteryx graminis*

TABLE 5

Observed feeds per minute of four common gulls, Unst

Gull 1	.	.	20 feeds in 7¼ minutes
Gull 2	.	.	3 ,, ,, 2 minutes
Gull 3	.	.	6 ,, ,, 3½ minutes
Gull 4	.	.	5 ,, ,, 5½ minutes

This shows an average of one feed per half minute.

were also among its stomach contents (plate I, fig. 1). Gull 2 contained 10 *A. glareosa* of which one also was *f. typica*. Two in 18 (11 per cent.) *A. glareosa* were therefore of the white form; the frequency in the local population was 2·76 per cent. The gull which was shot on the releasing ground contained one marked *typica* previously released by us. On being shot and held upside down the gulls regurgitated the insects which were at the time unrecognisable (plate I, fig. 2). These were cleaned in spirit by Graham Phillips and he was able to show that shortly afterwards both forms were easily identified; the black ventral surface of the abdomen of *f. edda* was distinguishable in the most mutilated specimens. Furthermore he observed the feeding habits of the gulls. They worked individually at a considerable distance apart, and as many as 25 could be seen at a time pacing the short heather on the hillside. He noted the number of feeds per gull per minute for birds which were actually feeding and not preening or resting. Table 5 gives the number of feeds for four common gulls under observation.

As the stomach contents contained by volume 60 per cent. of *A. glareosa* it is likely that during this period of the year this species is one, if not the main, food source of the common gull. Because of its size, many ounces per day must be eaten by each gull. Their efficiency in finding them astonished us because of the cryptic perfection of the moths, particularly that of *f. edda*. We ourselves succeeded in discovering only one *f. edda* at rest in the wild, though we were able to

follow many hundreds of released ones to their final resting positions (figured Kettlewell, 1961). Phillips also examined the stomach contents of wheatear, peewit and starling but no individual Lepidoperta were recognisable. However, under the microscope he showed that the stomach contents contained large quantities of Lepidopterous scales. As *A. glareosa* was the commonest species of moth, it is reasonable to assume that this species contributed to them.

It is certain that throughout the summer a high predation pressure on Lepidoptera is maintained by indigenous birds and that in August and September diurnal predation is increased in North Shetland due to the arrival of migrating flocks. These are unlikely to affect the south Shetland Mainland to the same extent. Under these conditions selective visual predation must be intense, particularly in the north.

(ii) *Evidence of* A. glareosa *predation by night.* In 1959 we observed birds, in particular the common gull and wheatear, feeding on the ground until late twilight during the first half of August. It is likely, especially in warm weather, that *A. glareosa* comes up and feeds on heather flowers during the long period of dusk. We have provided evidence (*vide ante*) that under these conditions *f. typica* would be at a greater cryptic disadvantage. During feeding, both forms frequently sit upside down beneath heather sprigs and, as distinct from *f. typica*, the black ventral surface of the abdomen of *f. edda* serves to make this form less conspicuous. We have as yet no evidence on the degree of crepuscular predation that takes place in this way, but it is our belief that, particularly when full moon falls in the first half of August, it is likely to be heavy. Extensive selective predation in fact may take place both by day and by night in certain years and under certain conditions.

B. Evidence of selective predation from mark-release-recapture data

We have shown from our day 1 and day 2 recapture data that a flight habit difference exists between southern *f. typica* and northern *edda*. This being so it is impossible in our present experiments to differentiate the results of increased dispersal from those of predation. Nevertheless the one must bear a relationship to the other. Under weather conditions such as those experienced on Shetland an increased flight dispersal on small isles and headlands must frequently lead to death from drowning. This situation certainly applies to our releasing site (2) on Unst where the sea lies a few hundred yards to the east. It is therefore worth while considering our recapture data in this light. On Unst a total of 4344 *A. glareosa* (*edda* 2260; *typica* 2084) were marked and released. Of the *f. typica*, approximately 90 per cent. had their origin in south Shetland. In spite of the deficiency in *f. edda* recaptures for day 1 the total recaptures were 132 *f. edda* to 95 *f. typica*. A chi-squared test for significance cannot, however, be directly applied to these figures because an equal number of releases was not undertaken on each day, but it can be applied to the

two expecteds, *i.e.* 123·125 and 103·875 which gives $\chi^2_{(1)} = 1{\cdot}40$, $P > 0{\cdot}2 < 0{\cdot}3$.

The recapture figures reflect a total of 440 days' survival for *f. edda* in the wild to 256 for *f. typica*. These recapture samples are, however, biased by small numbers of individuals which had survived for a long period of time, and this method of comparison is not therefore strictly valid.

A more refined method is to take the daily recaptures of both forms and compare these with the number expected, along with their variance using Smith's method (Clarke *et al.*, 1956). Table 4 shows that 132 *f. edda* were recaptured and 123·125 expected (a variance of 51·426, $P = > 0{\cdot}21$ and $< 0{\cdot}22$). There was not therefore a significant excess of *f. edda* recaptured. On the other hand, it suggests a 7 per cent difference in favour of *f. edda* on Unst, though because of the size of the variance this could be as high as 17 per cent advantage or as much as 5 per cent disadvantage. The size of the wild population into which we fed our marked individuals was too large for a significant selective predation difference to be shown by these methods, nor would it be easy to differentiate the dispersal of *f. typica* from selective predation against it.

By contrast the mark-release-recapture results in south Shetland, where no visible evidence of bird predation was seen, showed no differential survival. In releases totalling 2144 *A. glareosa* (*edda* 961; (approximately 92 per cent imported from Unst); *typica* 1183) observed *edda* recaptures were 50, expected 49·034. In the future therefore the answer to selective bird predation must depend, not on mark-release-recapture data, but on analyses of bird stomach contents, and for this the common gull has been shown to be the most suitable species.

7. CONCLUSIONS

The melanic form of *Amathes glareosa*, *f. edda*, is found at varying frequencies not only throughout Shetland and Fair Isle but also on the Orkneys. The polymorphism is likely therefore to be of considerable age and must be maintained by natural selection. A balance of advantages and disadvantages exists between the wild type and the melanic *f. edda*. By analysis and observation in the field we have been able to provide evidence as to what some of these are. The one gene difference between the two forms is responsible for the substitution of the normal disruptive pattern found throughout its range elsewhere, by a cryptic one of high efficiency for resting on the background of peat which is found throughout the northern half of Shetland. Under conditions of high selective pressures and in latitudes which permit many hours of both daylight and twilight predation, efficient camouflage has a priority over other demands.

Diminished flight activity, which may or may not be associated with *f. edda*, would also conserve loss through wind dispersal on small islands. On the other hand the advantages of flight and the normal

disruptive pattern in south Shetland, outweigh their disadvantages in north Shetland. This may in part be brought about by a relaxation of bird predation here, in a district of more cultivation, less peat-hag and with fewer small islands and headlands. In particular the common gull and also the vast flocks of immigrant birds from the north which arrive in north Shetland during the months of August and September are less frequent in the south. In Unst (and also Fair Isle), where gull predation is high, there is evidence that the light coloured *f. typica* emerge later in the hatching period than *f. edda*. The longer hours of darkness which rapidly come on later in the season, and the shorter periods of twilight, give more protection to this form, which we have shown is most conspicuous at dusk. Natural selection would therefore favour those *f. typica* which hatched later.

8. SUMMARY

1. By observation and experimentation an attempt has been made to analyse the relative advantages and disadvantages of melanism of non-industrial origin.

2. Mark-release-recapture experiments were conducted in both north and south Shetland on the moth *Amathes glareosa* Esp. A total of 7232 individuals of both forms were marked and released into the wild populations.

3. On Unst, north Shetland, where heavy predation was shown to take place by the common gull and other birds, a 7 per cent advantage of the melanic form over *f. typica* is indicated from our recaptures, though this is not significant.

4. In south Shetland where no predation was witnessed, there was no differential elimination disclosed from our recapture data.

5. An examination of the stomach contents of the common gull showed that in north Shetland, Lepidoptera, and in particular *A. glareosa*, formed one if not the chief source of food in the month of August. We have produced some evidence that they predate selectively.

6. A habit difference in which the melanic *f. edda* on Unst exhibit diminished flight activity compared to *f. typica* from south Mainland was demonstrated by three separate methods.

7. We have not yet been able to establish whether this difference in flight activity is associated with the *f. edda* or with the north and south populations.

8. In north Shetland and possibly Fair Isle, the wild type *A. glareosa* emerge later in the season than *f. edda*. Possible causes of this are discussed.

9. This polymorphism is almost certainly of considerable antiquity. It is maintained by a balance of colour, behavioural (and probably also physiological) differences. In the north efficient camouflage and diminished flight activity outweigh the advantages of the normal disruptive pattern and increased dispersal which are favoured in south Shetland as elsewhere in Britain.

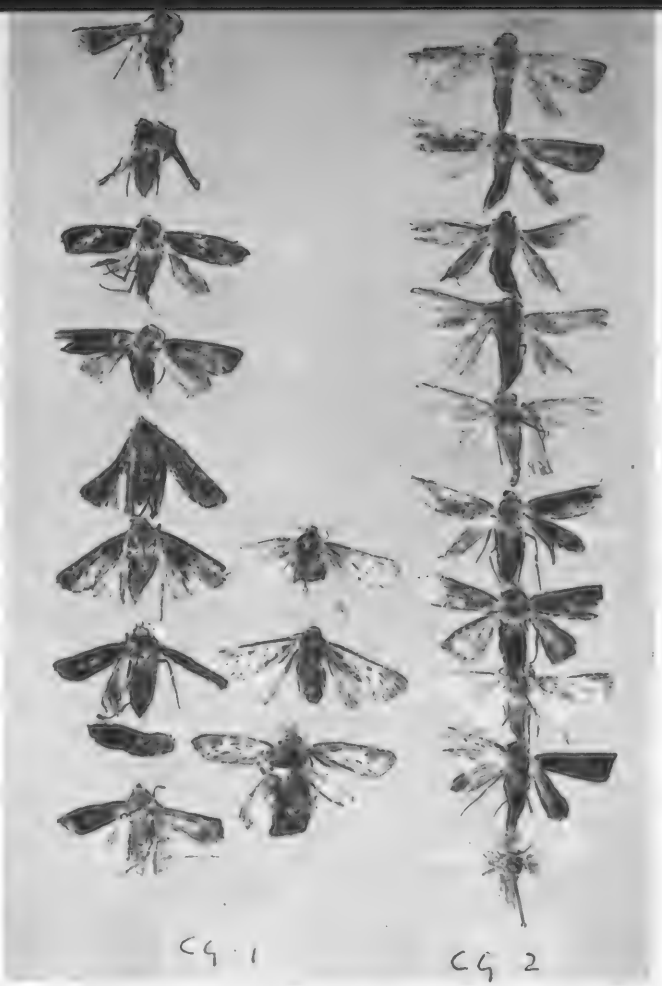


FIG. 1.—Stomach contents taken from two common gulls shot on Unst, 1960.

FIG. 2.—Common gull regurgitating *A. glareosa*, Unst 1960.

Addendum

This present work is being undertaken with the object of finding out the uses of melanism in the past. This might throw light on the origin of the present-day industrial melanics. I therefore decided to introduce a number of *f. edda* ova from Shetland of known gene-frequency into a circumscribed industrial area and to see if they subsequently behaved as industrial melanics. The oasis of Sutton Park, Birmingham, seemed ideally situated for this. *A. glareosa f. typica* is common here but no melanic forms occur. On 12th September 1960 we distributed 10,000 (±500) ova with a gene-frequency of approximately 84 per cent. *f. edda* onto the heather slopes of the Park. Due to the dark colouration of the ground it is likely that *f. edda* may have a cryptic advantage; theoretically also diminished flight activity may contain it within the bounds of this limited area. The analogy of Sutton Park to an island is not altogether misplaced. It will be of interest to see whether in the future *f. edda* survives and behaves as an industrial melanic in this area.

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SELECTION AND POPULATION STRUCTURE IN *LOLIUM*

V. CONTINUED RESPONSE AND ASSOCIATED CHANGES IN FERTILITY AND VIGOUR

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1. INTRODUCTION

EARLIER papers (Cooper, 1959*a*, *b*, *c*; 1960) have described the first three generations of response to selection for date of ear emergence in Irish and Kent perennial ryegrass (*Lolium perenne* L.). This character shows high heritability (>70 per cent.), most of the variation being genetic and additive. Rapid immediate response to selection occurred in both varieties, and by the third generation the extreme selection lines were well outside the parental range. Furthermore, most plants are highly heterozygous, and even one plant can carry considerable potential genetic variation.

Two further aspects which required investigation, (i) the pattern of response to continued selection over several generations and (ii) the effect of selection for ear emergence on other characters, particularly fertility and vigour, are dealt with in this paper.

2. MATERIAL AND METHODS

The foundation populations consisted of Irish and Kent perennial ryegrass from commercial stocks. Irish is an early-flowering variety which has been grown for seed production for many generations, while Kent is a late-flowering pasture variety, with little agronomic selection for either seed production or uniformity of heading (Cooper, 1959*a*). Ear emergence occurs in Irish about the beginning of May, and in Kent some 20-25 days later, but the exact date is greatly influenced by spring temperature, and year to year fluctuations of up to 14 days may occur (table 1). The phenotypic variance is usually greater in Kent than in Irish.

Operational details of the selection programme have been given by Cooper (1959*c*). During the first three generations, early and late selection lines based on one, two and four foundation plants were derived from each variety. Back-selection was started in the first generation to assess the amount of potential genetic variation carried within each initial group of plants. After the third generation, the lines derived from one and two initial plants showed pronounced inbreeding depression, and only the four-plant lines were continued.

Male and female fertility were measured in summer 1960 on the original varieties and the sixth generation selection lines. Male fertility was assessed from the percentage of pollen stainable with aceto-carmine. Anthers were fixed in 70 per cent. alcohol just before anthesis, and counts were made on anthers from three heads on each plant to give an estimate of within-plant variation. Pollen stainability does not strictly measure the viability of the pollen, but it does provide an upper limit for fertility. Female fertility was measured as the percentage seed-set from open-pollination in the field. The selection experiment was sited in the middle of the plant breeding nursery, so that availability of pollen was not likely to have been

limiting. Three heads were taken from each plant to provide a measure of within-plant variation.

Since the selection programme involves a moderate degree of inbreeding, which may itself have an effect on fertility and vigour, an attempt was made to determine the actual level in the selection lines by comparison with families of known degrees of inbreeding. The original varieties and the first five generations of selection were

TABLE 1

Date of ear emergence in the original varieties

Year	Irish		Kent	
	Mean	Var.	Mean	Var.
1954	3·4 May	24·0	24·1 May	26·4
1955	14·0 ,,	30·7	29·7 ,,	24·5
1956	8·7 ,,	12·4	22·5 ,,	18·0
1957	30·4 April	18·7	22·8 ,,	40·0
1958	8·3 May	17·5	28·2 ,,	25·3
1959	30·1 April	27·8	21·5 ,,	17·5
1960	30·0 ,,	17·0	23·5 ,,	41·4
Mean	5·0 May	21·2	24·6 May	27·6

sown in a warm glasshouse in January 1960, together with several sib-crosses and selfed progenies of Irish and Kent ryegrass. The following seedling measurements were taken :

(i) *leaf size*, measured as length × median width of the sixth leaf on the main shoot
(ii) *rate of leaf appearance*, from the 4th to the 7th leaf on the main shoot
(iii) *tiller number, dry weight per plant and per tiller* measured 78 days after sowing.

The genetic control and inter-relationship of these seedling characters have been described elsewhere (Cooper and Edwards, 1961).

3. RESPONSE TO SELECTION

(i) *Change in means*

The pattern of response to selection is shown in fig. 1 where the cumulative response for each generation is plotted against the cumulative selection differentials, as suggested by Falconer (1955). The numerical values for selection differentials, response and realised heritabilities are given in table 2. The release of variation from within each of the initial four-plant groups is shown in fig. 2, and the total range achieved in table 3. The response in each generation has been measured as the difference between the mean of the selection line and that of the original variety in the same year.

Response has continued at a linear rate in most of the forward lines. The extreme lines are now well outside the limits of the original varieties and the full range of Irish has been obtained from Kent, and

vice versa. Although the selection differential is similar in both directions, the response, and, therefore, the realised heritability, are asymmetrical, being always greater in the late direction. Both the selection differential and the heritability are greater for corresponding lines in

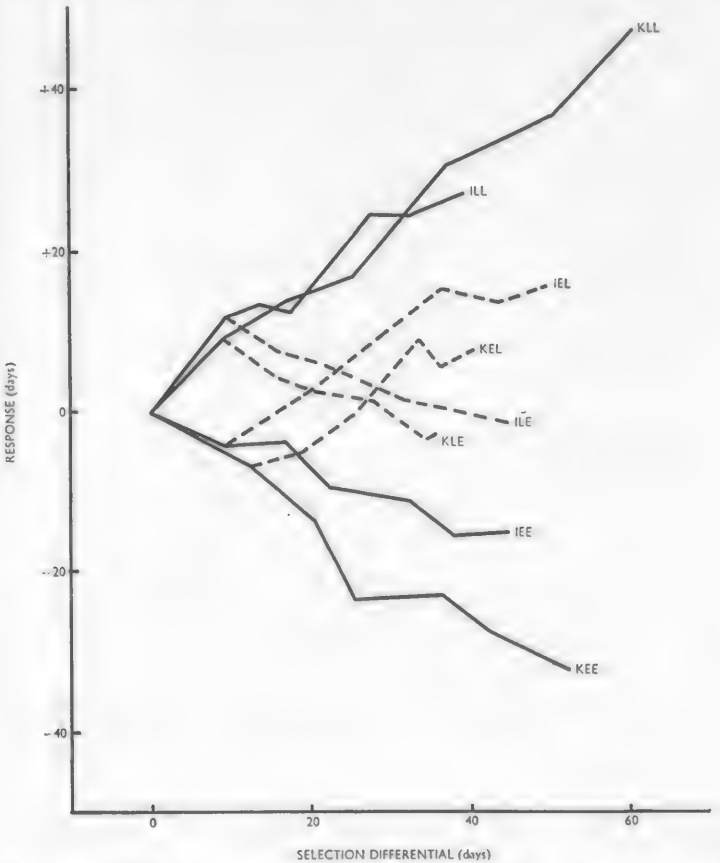

FIG. 1.—Response to selection for early and late ear emergence. Irish and Kent perennial ryegrass.

KEE =	Kent early-early	IEE =	Irish early-early
KEL =	,, early-late	IEL =	,, early-late
KLE =	,, late-early	ILE =	,, late-early
KLL =	,, late-late	ILL =	,, late-late.

Kent than in Irish, possibly as a result of less stringent agronomic selection in the past. The realised heritabilities are quite high (30-70 per cent.) and there is no marked evidence as yet of any ceiling of response having been reached, except perhaps for the earliest line

(IEE). Even so, the realised heritabilities are below the estimates obtained from parent-progeny regression in one year, particularly in the early lines. This may be due partly to genotype-environment

TABLE 2

Selection differentials and realised heritabilities in the selection lines

	Selection differential (days)	Response (days)	Realised heritability (Per cent.)
Forward selection (6 *generations*)			
Irish Early (EE) . .	−45·0	−15·0	33·3
Late (LL) . .	+39·6	+27·1	68·4
Total	84·6	42·1	49·8
Kent Early (EE) . .	−52·6	−32·3	61·6
Late (LL) . .	+60·7	+47·5	78·3
Total	113·3	79·8	70·6
Back selection (5 *generations*)			
Irish Early-late (EL) .	+39·7	+19·5	49·1
Late-early (LE) .	−35·5	−13·2	37·2
Kent Early-late (EL) .	+28·3	+14·1	49·8
Late-early (LE) .	−26·7	−12·0	44·9
Release from within four-plant groups (5 *generations*)			
Irish Early (EE-EL) . .	75·3	30·4	40·4
Late LE-LL) .	65·5	28·3	43·2
Kent Early (EE-EL) . .	68·1	39·9	58·6
Late (LE-LL) . .	78·2	50·4	64·5

TABLE 3

Phenotypic range in the original varieties and sixth generation selection lines

Original variety		Six generation selection lines	
Mean	Range	Range between means	Range between extreme plants
Irish 30·0 April .	17 April-11 May (24 days)	15·8 April-27·9 May (42·1 days)	28 March-9 June (73 days)
Kent 23·5 May .	8 May-6 June (29 days)	21·2 April-10·0 July * (79·8 days)	7 April-6 Aug.* (121 days)

* Omitting 3 non-flowering plants.

interaction from year to year, and partly, as will be discussed later, to inbreeding depression in the late lines resulting in asymmetrical response.

In general, the response in the back-selection lines has not been so great as in the forward lines, and in two lines (KEL and KLE) low

fertility has severely decreased the possible selection differential in the later generations. Even so, the release of variation from within four initial plants of the same phenotype has been considerable (table 2 ; fig. 2), and the early and late lines from the same initial group are by now completely discontinuous. The release of variation is again less

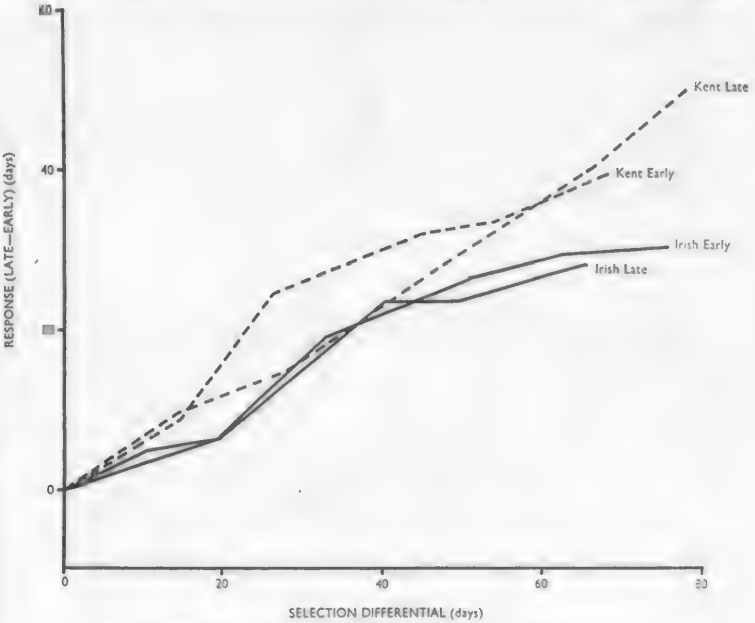

FIG. 2.—Release of variation from foundation groups of four plants.

in Irish than in Kent, but there is no regular difference between the early and late foundation groups.

(ii) *Change in variances*

The changes in phenotypic variance within the lines during the course of selection are shown in table 4, which also gives the within-genotype variance derived from clonal replicates in each generation. In the original varieties, Kent usually shows a higher phenotypic variance than Irish. In the first generation the variance decreases as expected, since it is based on pair-crosses with strict assortative mating for date of ear emergence. There is no regular change in subsequent generations, and the average within-line variance in the sixth generation approaches that of the original varieties. There are, however, marked differences between lines, and by the sixth generation the back-selection lines are usually more uniform than the forward lines. This is

particularly marked in lines IEL and KEL, and the possible selection differential is correspondingly reduced (fig. 1).

In spite of the selection and inbreeding there has been no regular change in the developmental error within clones. A surprisingly high

TABLE 4

Changes in variance for date of ear emergence under selection

	Original varieties						Mean
	1955	1956	1957	1958	1959	1960	
Irish	30·7	12·4	18·7	17·5	27·8	17·0	20·7
Kent	24·5	18·0	40·0	25·3	17·5	41·4	27·8
	Generations of selection						
	1st (1955)	2nd (1956)	3rd (1957)	4th (1958)	5th (1959)	6th (1960)	
Irish							
EE	19·8	13·2	21·9	12·1	19·9	61·6	
EL		17·2	29·7	33·3	8·3	7·8	
LE	7·4	13·3	15·8	12·1	24·1	12·3	
LL		9·8	18·8	7·8	16·4	30·7	
Mean	13·6	13·4	21·6	16·3	17·2	28·1	
Kent							
EE	15·5	10·2	24·2	13·2	32·8	50·8	
EL		14·5	12·3	25·1	5·0	7·5	
LE	16·4	12·1	15·7	13·7	15·5	13·3	
LL		33·2	10·0	44·9	18·0	(123·7)*	
Mean	16·0	17·5	15·6	24·2	17·8	23·9	
Developmental error							
Irish	1·7	2·7	1·4	5·3	4·8	...	
Kent	1·7	4·0	2·9	7·1	1·6	...	
Mean coeff. of inbreeding	0	0·125	0·219	0·305	0·381	0·449	

* Three plants failed to head. This variance, based on heading plants only, not included in mean.

figure recorded for both Irish and Kent in the fourth generation, but this is not continued in later generations. Thus, there appears to be no regular decline in genotypic variation within the lines, in spite of the expected effect of selection in concentrating genes for early and late ear emergence, and the moderate inbreeding practised, which gives a theoretical inbreeding coefficient of 0·45 by the sixth generation.

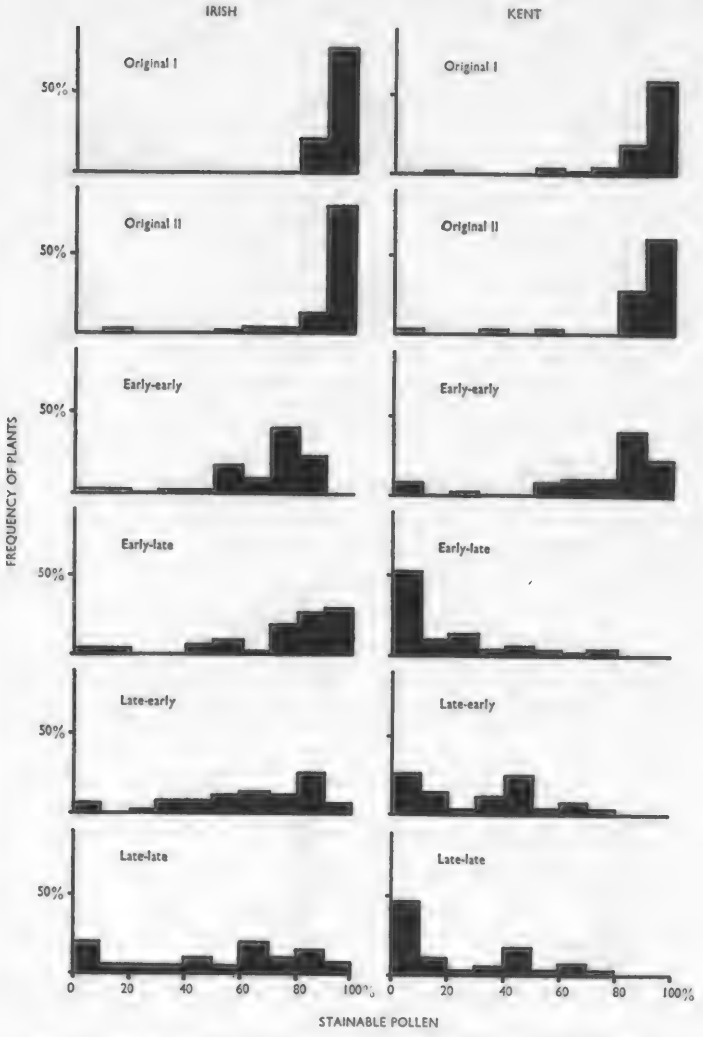

FIG. 3.—Male fertility in original varieties and sixth generation selection lines.

4. CHANGES IN FERTILITY

The male and female fertility in the original varieties and in the sixth generation lines are shown in figs. 3 and 4 and the between- and within-plant variances are given in table 5. All fertility data were transformed to degrees for statistical analysis.

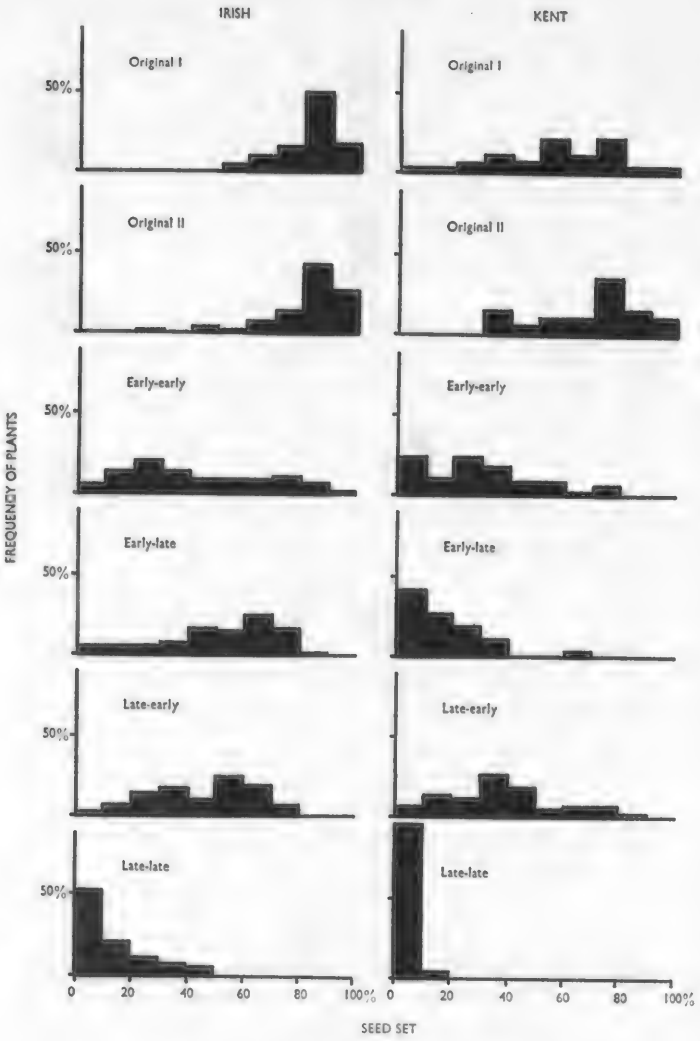

FIG. 4.—Female fertility in original varieties and sixth generation selection lines.

The pollen fertility in the two samples of each original variety was high, being over 90 per cent. in most plants. Kent, however, has a lower mean and greater variance than Irish, with a marked tail of plants of low fertility. The within-plant variance is also higher in Kent than in Irish.

All the selection lines show a reduction in pollen fertility, with an increased variance, though the early lines are usually more fertile than the late, possibly due to a lower degree of inbreeding. There is little difference between Irish and Kent for corresponding forward selection lines, but the back selection lines KEL and KLE are very low (12 per cent. and 15 per cent.). These low pollen fertilities result

TABLE 5

Male and female fertility in the selection lines

	Irish				Kent			
	Mean (Per cent.)	V_P between plants	V_E within plants	No. of plants	Mean (Per cent.)	V_P between plants	V_E within plants	No. of plants
Pollen stainability								
Original .	93·0	10	11	49	91·1	108	18	48
	92·2	92	11	48	87·5	198	20	26
EE . .	69·7	131	31	46	76·6	324	53	50
EL . .	76·3	296	15	47	12·1	320	10	48
LE . .	60·7	358	81	43	14·9	415	55	33
LL . .	49·1	506	80	41	46·0	388	295	24
Seed set								
Original .	81·7	137	76	49	56·0	227	175	49
	83·2	184	54	48	69·7	92	130	24
EE . .	43·1	296	127	41	24·1	284	99	44
EL . .	53·1	213	48	40	13·3	163	69	37
LE . .	37·1	131	75	48	37·8	80	239	41
LL . .	8·2	51	188	36	<1·0	6	■	24

NOTE.—All variances based on data transformed to degrees

Sample analysis	Pollen stainability (IEE)			
	*d*F	SS	MS	Expectation
Between plants .	45	19,124	425	V_E+3V_P
Within plants .	89	2,727	31	V_E
Total . .	134	21,851		

in non-dehiscent anthers and, therefore, functional male sterility in many plants, but are not accompanied by a corresponding decrease in female fertility. The within-plant variance is increased in most of the selection lines.

Female fertility presents a similar picture though the figures are lower than for pollen fertility (except for the male-sterile lines KEL and KLE). The seed-set for the original Irish is high, usually over 80 per cent., but in Kent the mean is lower (50-70 per cent.) with a

larger variance, suggesting less stringent agronomic selection in the past. All the selection lines show a much lower seed-set than do the original varieties, and this is most marked in the late lines (ILL and KLL). In KLL, which is very late flowering, this may result from lack of available pollen, but for ILL ample pollen was present. The Kent lines again usually show a lower seed-set than the corresponding ones from Irish and, in the forward selection, the early lines are usually more fertile than the late.

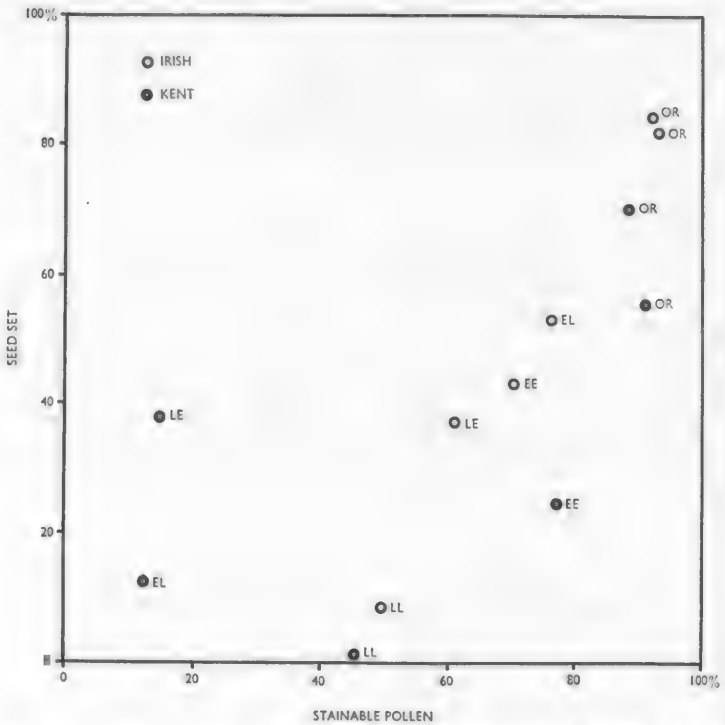

FIG. 5.—Correlation between mean male and female fertility in original varieties and sixth generation selection lines.

There is a good correlation between *mean* seed-set and pollen stainability for the original varieties and the selection lines (fig. 5), with the exception of KEL and KLE, which were noted as early as the third generation to show poor pollen fertility and a high proportion of non-dehiscent anthers. The reduction in seed-set is greater than that in pollen fertility, but the two are roughly proportional, which suggests a similar mechanism operating in the two sexes.

The correlation of male and female fertility *within* the lines, however, is more complex (fig. 6). With high pollen fertilities above about

50 per cent., there appears to be no correlation with female fertility, but when the pollen stainability falls below 50 per cent. a marked correlation with seed-set becomes apparent, and very low pollen fertilities are usually accompanied by low seed-sets. This correlated

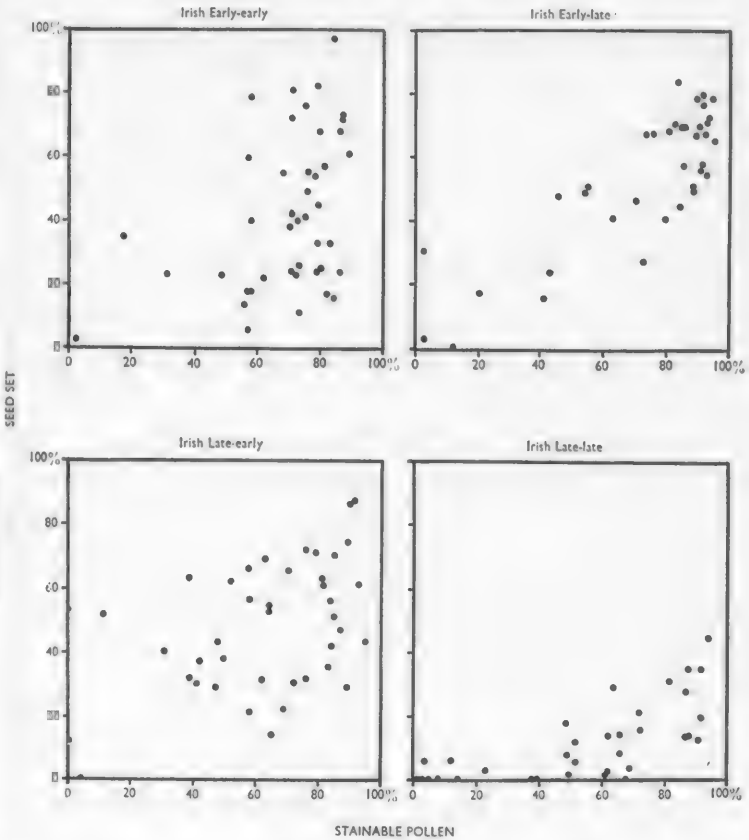

FIG. 6.—Correlation between male and female fertility *within* selection lines of Irish perennial ryegrass.

effect on seed-set is not a result of shortage of pollen, as seed fertility was assessed from open-pollination in the breeding nursery.

The decreased fertility obtained as a result of selection for early and late ear emergence suggests that the extremely early and late plants within each line might be obviously less fertile. The correlation diagrams of male and female fertility against date of ear emergence were therefore plotted for the original varieties and the sixth generation selection lines, but, rather surprisingly, the association between

fertility and the extremes of ear emergence was slight and *within* the late lines only (fig. 7).

The present selection programme for ear emergence evidently leads to a decline in both male and female fertility, but the relative

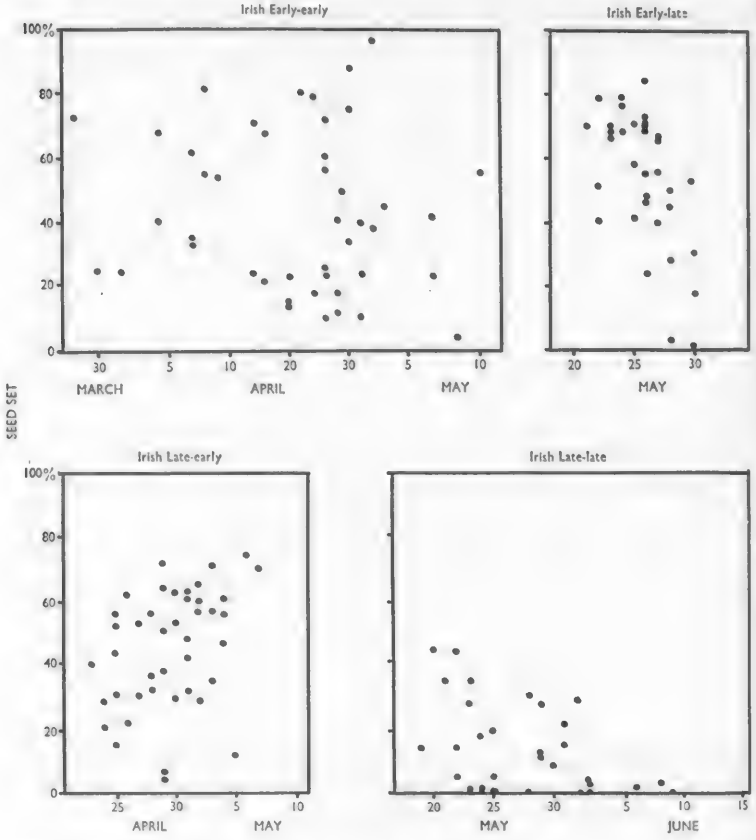

FIG. 7.—Correlation between female fertility and date of ear emergence *within* selection lines of Irish perennial ryegrass.

roles of selection and inbreeding in producing this effect are difficult to separate.

5. SEEDLING CHARACTERS

The seedling results should throw light on two important points: first, the extent of any correlated response to selection for ear emergence, and second, the actual degree of inbreeding reached in the selection lines.

The seedling measurements for the original varieties and the fourth and fifth generation selection lines are given in table 6, while the behaviour of these characters under controlled inbreeding *without* selection is shown in table 7.

TABLE 6

Correlated response and degree of inbreeding in seedling characters

	Irish				Kent*			Sig. diff. between means at 5 per cent.
	Original	Means		Diff. between lines in 5th gen.	Original	Means		
		4th gen.	5th gen.			4th gen.	5th gen.	
Leaf size (mm.²) .	1076	1071	1082	EL>EE	841	861	832	97
Rate of leaf appearance (days per leaf)	7·3	7·5	8·0	EL>EE	7·0	7·1	6·9	0·33
Tiller No. . .	18·5	16·6	16·0	LE>LL	17·3	13·0	12·3	2·3
Dry weight per plant (gm.)	0·55	0·49	0·44	None	0·44	0·34	0·33	0·08
Dry weight per tiller (mgm.)	31	30	29	None	25	28	27	3·5
Mean coefficient of inbreeding	0·0	0·305	0·381	...	0·0	0·305	0·381	...

* No significant differences between pairs of selection lines for Kent in the 5th generation.

TABLE 7

Effects of inbreeding without selection on seedling characters

		Orig. variety	Outcross	Sibcross	Second sibcross	Selfing	Change per 10 per cent. inbreeding
Leaf size (mm.²) .	Irish	1076	1056	1002	889	790	51·0±17·9**
	Kent	841	1002	901	879	464	48·7±28·5 ns
Rate of leaf appearance (days per leaf)	Irish	7·3	7·1	7·6	7·7	7·3	0·003±0·093 ns
	Kent	7·0	7·2	7·4	7·3	7·6	0·083±0·049 ns
Tiller No. . .	Irish	18·5	19·8	15·6	13·9	14·2	1·20±0·22***
	Kent	17·3	15·9	14·6	13·3	13·2	0·76±0·28*
Dry weight per plant (gm.)	Irish	0·55	0·59	0·44	0·35	0·33	0·54±0·006***
	Kent	0·44	0·47	0·38	0·33	0·21	0·040±0·015*
Dry weight per tiller (mgm.)	Irish	31·0	30·6	28·3	25·7	23·8	1·36±0·476**
	Kent	25·5	27·8	27·3	25·5	16·0	0·95±0·762 ns
No. of samples . .	Irish	2	8	8	6	6	
	Kent	2	4	7	2	1	
Coefficient of inbreeding		0	0	0·25	0·375	0·50	

There is no regular pattern of correlated response. Out of the 20 possible comparisons (late-early) in the fourth generation, only one (leaf size in Kent) shows a significant difference, while in the fifth generation only 3 differences (all in Irish) are significant. Furthermore, the differences are not always in the same direction. This lack of regularity is similar to that previously reported for other ear characters, such as ear length and number of spikelets (Cooper, 1960) and suggests that ear emergence and seedling characters could be selected independently.

It now remains to assess the mean inbreeding depression in the selection lines, using these seedling characters as standards. As table 7 shows, all the seedling components, except rate of leaf appearance, show inbreeding depression at a roughly linear rate. A comparison of the fourth and fifth generation selection lines with the original varieties, however, shows that in only 5 out of 10 instances was a significant depression obtained in seedling characters. There was no evidence of a greater degree of inbreeding depression in the late selection lines, although these might be expected to be more homozygous. The degree of inbreeding required to produce these effects in the selection lines has been calculated, and in nearly all cases the actual degree of inbreeding is considerably less than that expected on theoretical grounds. No deliberate selection for vigour was made in the seedling stage, and any natural selection for heterozygotes must have operated before germination, either through differential fertility of the parents, or during early seed development.

It is clear, therefore, that the seedling characters, although known to show inbreeding depression, do not appear to have been greatly influenced by selection for ear emergence.

6. DISCUSSION

The genetic structure of these varieties of ryegrass is evidently similar to that of other outbreeding species in that most individuals are highly heterozygous and can carry much potential genetic variation. The amount of variation and its rate of release will depend on two factors, the past selection history of the character, and its relation to fitness (Robertson, A., 1955).

(i) *Response to selection*

The pattern of short-term response for ear emergence is similar to that for other characters showing largely additive genetic variation, such as body weight and bristle number in *Drosophila* (Mather, 1953; Robertson, F. W., 1955; Clayton, Morris and Robertson, 1957). Response is still continuing in the sixth generation and the means of the selection lines go well outside the limits of the original varieties. There are, however, regular varietal and directional differences in the pattern of response.

The selection differential, the response and the realised heritability are much greater in Kent than in Irish for all selection lines. Irish ryegrass has been exposed to selection for uniform flowering during more than 70 generations of seed multiplication, although a range of 20-25 days is possible within any seed crop (Cooper, 1959*a*). Kent ryegrass, on the other hand, is derived from old pastures which have been grazed for many decades, and the commercial seed is only a few generations removed from these old pastures. Apart from the effects of heavy spring grazing, there has been no selection for uniformity of flowering in the original sward, although three or four generations of seed multiplication may well have limited the phenotypic variation present. Kent ryegrass has thus been exposed in the past to less stringent selection for flowering time than Irish, which may account for its more rapid release of variation.

The other marked feature is the asymmetrical response in the late and early lines. Possible causes of such asymmetry have been discussed by Falconer (1955). In the present case, the selection differential is similar in the two directions, which leaves directional dominance (inbreeding depression) or unequal gene-frequencies as possible explanations.

Date of ear emergence shows some inbreeding depression, selfing resulting in a delay of about 6 days (Cooper, 1959*b*), so presumably some directional dominance exists. Selection would therefore be expected to progress more rapidly in the late direction, because of the selection of more homozygous plants in the late lines (Falconer, 1955), although the recorded asymmetry would require a greater degree of inbreeding than appears to be present in the selection lines. There is no evidence on the relative gene frequencies in the two directions, but selection in the past has probably favoured an intermediate expression of the character.

So far no marked ceiling of response has been reached, but this would not be expected on genetical grounds as early as the sixth generation (Falconer, 1955; Clayton, Morris and Robertson, 1957). Response has continued steadily in most lines, and the range between the extreme lines is now 8 to 15 times the original phenotypic standard deviation. There is still appreciable phenotypic variation within most lines (table 4) though this does not necessarily mean that response to selection can still be expected (Robertson, F. W., 1955).

A physiological limit has, however, been reached in the extreme early line (from Irish) and in the extreme late line (from Kent). Ear initiation in *Lolium* in the spring is a response to long photoperiod, but further elongation and development of the inflorescence are strongly influenced by spring temperature (Cooper, 1959*a*). In the late Kent line, a few non-heading plants have appeared in the sixth generation, possibly because of a high photoperiod requirement. In the early Irish line, on the other hand, although the photoperiod requirement is low, the earliest plants may be initiating heads at a date when spring

temperature is too low for any active growth. Furthermore, since ear emergence in the early lines is a complex response to temperature and photoperiod, selection in a warm spring could operate for a low critical photoperiod, but in a cold spring, temperature might be limiting, resulting in appreciable genotype-year interaction. Such interaction may explain the low realised heritability in the early selection line from Irish (table 2).

The main limit to selection so far has been low fertility and therefore attenuated selection pressure in the lines KEL and KLE. Since only 50 seedlings of each line are required in each generation to continue the selection programme, moderate infertility has not been selected against.

(ii) *Changes in fertility and vigour*

Selection for a single character has frequently been reported to produce associated reductions in fertility and viability (Mather and Harrison, 1949; Lerner and Dempster, 1951; Clayton, Morris, Knight and Robertson, 1957) though low fertility does not usually become a problem until after 15 to 20 generations. The usual explanation of such decline is the unbalancing effect of selection for one character on closely-linked genes controlling fertility and viability. In addition, most selection experiments involve some degree of inbreeding, which itself would be expected to unbalance fertility in a normally outbreeding population (Rees, 1955).

In the present case, fertility is high in both original varieties, though less so in Kent, which has presumably undergone less stringent selection for seed production in the past. In fact, Davies (1954), in a survey of potential breeding material from old pastures found a range of 12 per cent. to 76 per cent. with a mean of 42 per cent., suggesting little selection for seed fertility under these conditions. By the sixth generation, however, both male and female fertility have fallen markedly, usually more in Kent than in Irish, and more in the late lines than the early lines. Even so, most lines contain a range of values, and selection for high fertility should be possible.

This rapid decline in fertility may be the result either of selection or of the accompanying inbreeding, which may be quite high (the theoretical inbreeding coefficient is 0·45 by the sixth generation). The evidence from the seedling characters, however, indicates that the actual degree of inbreeding is considerably less than the theoretical, and this is also borne out by the maintenance of genotypic variation in the selection lines.

The lack of unbalance in the seedling characters suggests either that fertility is more sensitive than seedling vigour, or that some developmental (rather than strictly genetical) relationship with ear emergence is involved. Cytological analysis to be reported later shows that in the selection lines the timing of meiosis is unbalanced relative

to ear emergence, being advanced in the late lines and retarded in the early ones.

Further work is evidently necessary to separate the effects of inbreeding from the *genetical* unbalance caused by selection and from the *developmental* unbalance resulting from abnormal timing of meiosis. The possibilities of selecting for higher fertility in these lines is also important.

(iii) *Plant breeding implications*

In these ryegrass varieties, rapid and extensive response to selection has been obtained for one character, date of ear emergence, without correlated change in other ear characters, or in seedling components of vigour, though with a decrease in fertility. Is such response likely to be possible for other agronomic characters, and what are the dangers of an associated decline in fertility?

As Robertson, A. (1955) points out, the pattern and extent of response to selection in a character will depend on its previous selection history and on its relation to fitness. If a character is closely related to fitness, as for egg-laying in *Drosophila* and probably fertility in ryegrass, selection will have favoured a maximum expression in the past, and little additive genetic variation will be left. Furthermore, if selection is relaxed the character may well decline in fitness. If, however, a character has been selected for intermediate expression, as for date of ear emergence in ryegrass and possibly body size in *Drosophila*, appreciable additive genetic variation may remain, although there may be some inbreeding depression. In the extreme case, where a character appears to be peripheral to fitness, as in chaeta number in *Drosophila*, much additive genetic variation should occur and response to selection should be rapid and extensive.

In ryegrass and other herbage grasses, therefore, one would expect immediate components of fitness, such as survival under competition in a pasture sward, or fertility in a seed crop, to have been already selected for maximum expression and show little additive genetic variation and response to selection. On the other hand, characters which do not contribute to survival in the field, such as nutritive value and certain biochemical and mineral components (Vose, 1960) may retain useful additive genetic variation, provided they possess no negative genetic correlation with fitness.

This argument may not seem at first sight to offer much hope to the plant breeder for the direct selection of major components of fitness. It must be remembered, however, that even such major components as competitive ability and seasonal pattern of growth have been selected for optimum expression in one particular environment only. There appears for instance, to have been little or no selection for seed fertility in old pastures. Transfer of the population or variety to a different environment may uncover useful additive genetic variation. As shown earlier (Cooper, 1959*a*), ryegrass populations which are uniform in

flowering behaviour in their native environment may diverge greatly when transferred to others, and similarly those plants which show greatest competitive ability under intense defoliation do not necessarily produce most herbage under lenient cutting (Cooper, unpublished). Clausen and Hiesey (1958) have shown that the introduction of adapted ecotypes into other climatic environments may reveal a wealth of newly available variation, and the same may well be true for changes in agronomic management.

Work has, therefore, been started in ryegrass to assess the amount of additive genetic variation and response to selection for a number of agronomic characters, paying special attention to their past selection history, their relation to fitness and their expression in different environments (Cooper and Edwards, 1961).

7. SUMMARY

1. Selection for early and late ear emergence has been continued for six generations in Irish and Kent perennial ryegrass and associated changes in fertility and seedling components of vigour have been recorded.

2. Response to selection has carried all lines well outside the limits of the original varieties, and by the fourth generation the full range of Irish had been obtained from Kent and *vice versa*. Back-selection, which was started in the first generation, indicates that much potential genetic variation can be carried within four foundation plants of similar phenotype.

3. Both the possible selection differential and the realised heritability are greater in Kent than in Irish, possibly because of less stringent agronomic selection in the past. Response was asymmetrical in both varieties, being more rapid in the late direction, perhaps in part because of inbreeding depression in the late lines.

4. Male and female fertility were high in the original varieties but had declined in all lines by the sixth generation, in some cases severely reducing the possible selection differential. The variation in fertility between plants was increased in the selection lines, giving possibilities of future selection for high fertility.

5. Although most seedling characters, such as leaf size, tiller number and dry weight, show marked inbreeding depression, there is no invariable decline in these characters during the selection programme, and the actual degree of inbreeding is rather less than that expected on theoretical grounds. There is no regular correlated response in any of the seedling characters to selection for ear emergence.

6. These results are discussed in relation to the past selection history of the varieties, and it is concluded that a similar pattern of response might be expected for other agronomic characters, which are not immediate components of fitness.

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FLY SIZE, EMERGENCE TIME AND STERNOPLEURAL CHAETA NUMBER IN *DROSOPHILA*

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1. INTRODUCTION

In the literature, there are observations in *Drosophila melanogaster* on the effect of environment on chaeta number. For example, Plunkett (1926) studied various genes affecting chaeta number, and showed that under conditions of increased competition, chaeta number was reduced. Similarly, as the temperature at which flies were grown was increased from 14° C. to 30° C., a general decrease in chaeta number occurred. He found no effect in his wild-type stock, but, as his initial experiments were done on a small number of chaetæ on each side of the fly, this is not surprising. More recently, data on sterno-pleural chaeta number have been collected at 20° C., 25° C., 30° C. and a fluctuating temperature running smoothly from 30° C. at midday to 20° C. at midnight, and back to 30° C. at midday (Thoday, 1958, quoting Beardmore) for the F_1 and subsequent generations from Oregon and Samarkand inbred lines. At 20° C., chaeta number was higher than at 25° C., which in turn was higher than at 30° C. The fluctuating temperature (20/30° C.) gave about the same number of chaetæ as at 25° C., as might be expected. After the flies were placed in their new environment, further changes occurred in subsequent generations. At 20° C., chaeta number increased further, and at 30° C. decreased further. This progressive change from generation to generation was taken to indicate that chaeta number is a character of adaptive significance. Similarly, sternopleural chaeta asymmetry decreased over the generations in the 30° C. and 20/30° C. environments, this suggesting that sterno-pleural symmetry is of adaptive significance.

Further evidence that sterno-pleural chaeta number is of adaptive significance is presented by Mather (1953), Tebb and Thoday (1954), Thoday (1955) and Beardmore (1960), and in other publications.

It seems, therefore, reasonable to report some results for flies grown at two levels of competition on various concentrations of the tyrosinase inhibitor phenyl-thio-carbamide (P.T.C.) at 25° C. P.T.C. was shown by Parsons and Kroman (1960) to reduce the size of flies, and appeared to have no other morphological effect. An additional smaller experiment will be reported where flies grown at 30° C. are compared with those grown at 25° C. Thus it is possible to study relations between fly weight, which is a measure of fly size, and sterno-pleural chaeta number by growing flies on increasing concentrations of P.T.C. and at

different temperatures. That there is probably some relation between fly weight and chaeta number is indicated by Reeve and Robertson's (1954) results on sternite chaeta number where they found that the male fly had fewer chaetæ than the female, and the reduction seemed to be related to the surface area of the fly, which is of course related to fly weight. This sex difference applies to sternopleural chaetæ also (Mather, 1953). Reeve and Robertson (1954) also found a correlation between fly size, as measured by thorax length, and chaeta number for flies grown under variable culture conditions. Mann (1923) found that flies carrying the gene sternopleural that developed slowly had fewer chaetæ than those developing more rapidly, and that the reduction in chaeta number was related to fly size. Preliminary results showing a correlation between chaeta number, surface area, and fly weight for various genotypes under different environmental conditions have been recently reported (Gibson, Parsons and Spickett, 1961).

2. METHOD

Various numbers of replicates in 4″ vials were set up for the three genotypes, Oregon−R (++), ebony″ ($e''e''$), and Oregon×ebony heterozygotes ($e''+$) at 2 levels of competition, namely 25 and 100 newly-hatched larvæ per vial on a series of concentrations of P.T.C. at 25° C. For brevity, we shall refer to these 2 levels of competition as low and high levels. At high concentrations of P.T.C., the number of replicates was increased, in an attempt to obtain adequate data for meaningful results, since fewer flies were expected to emerge. The adults were scored daily as they emerged for fly weight and sternopleural chaeta number. A torsion balance accurate to 0·02 mgm. was used to measure fly weight.

A second smaller experiment was set up at 25° C. and 30° C. using the Oregon stock on a few levels of P.T.C., to obtain information on the effect of temperature on fly weight and chaeta number. In this experiment the high level of competition had 150 larvæ per replicate, and the low level 25 larvæ per replicate.

3. FLY WEIGHT AND P.T.C. CONCENTRATION

In fig. 1 graphs of mean fly weight are given for each sex and each level of competition, for the 3 genotypes ++, $e''+$, and $e''e''$ on various concentrations of P.T.C. The points on these graphs have necessarily different accuracies, depending on the number of flies classified. However, they are adequate to show any trends. A few points at the highest concentrations are omitted since very few flies emerged, and would confuse any trends. All the points plotted are means from 5 or more flies.

These graphs show clearly that (1) females weighed more than males at a given level of competition on a given concentration of P.T.C., (2) flies grown at the high level of competition weighed less than those grown at the low level for a given sex on a given concentration of P.T.C., and (3) fly weight decreased as P.T.C. concentration was increased. The greatest decrease in fly weight occurred on low concentrations of P.T.C. For $e''e''$, most of the decrease occurred between the control and 0·01 per cent. P.T.C. At 0·05 per cent. P.T.C., no $e''e''$ flies survived as found by Parsons and Kroman (1960).

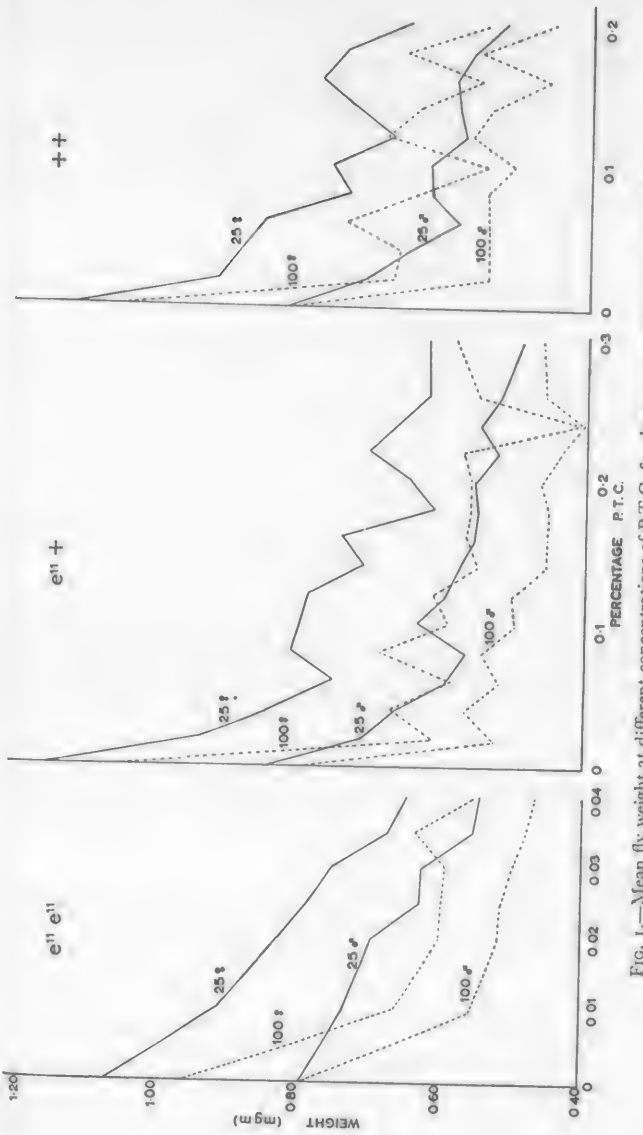

FIG. 1.—Mean fly weight at different concentrations of P.T.C. for the genotypes $e^{11}e^{11}$, $e^{11}+$, and $++$.

For $e''+$ and $++$, most of the decrease occurred between the control and 0·04 per cent. P.T.C. In this case, the lethal concentration was usually somewhat greater than 0·30 per cent. P.T.C. There was, however, a general tendency for fly weight to be slowly reduced at the higher concentrations of P.T.C. for all genotypes.

Coefficients of variation of fly weight were computed for some of the lower concentrations of P.T.C. (table 1). To avoid confusion, coefficients of variation are not given for P.T.C. concentrations where some, or all, of the entries in the table would be based on less than 30 flies.

TABLE 1

Fly weight and chaeta number variability

Larvæ per replicate	Fly weight coefficient of variation				Chaeta no. coefficient of variation				Chaeta no. asymmetry $\left(\frac{A}{T}\times 1000\right)$			
	25		100		25		100		25		100	
$e''e''$	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control	0·061	0·058	0·061	0·069	0·067	0·100	0·092	0·103	56	58	53	76
0·01 per cent. P.T.C.	0·102	0·077	0·081	0·120	0·084	0·082	0·104	0·107	57	51	65	35
0·02 per cent. P.T.C.	0·096	0·099	0·167	0·136	0·110	0·090	0·101	0·083	68	56	55	61
$e''+$												
Control	0·085	0·078	0·078	0·071	0·084	0·088	0·095	0·101	54	60	56	56
0·02 per cent. P.T.C.	0·113	0·083	0·110	0·114	0·081	0·094	0·073	0·097	56	54	45	49
0·04 per cent. P.T.C.	0·147	0·110	0·158	0·134	0·094	0·092	0·091	0·114	63	53	57	59
0·08 per cent. P.T.C.	0·123	0·170	0·173	0·149	0·081	0·088	0·097	0·087	59	46	42	59
0·10 per cent. P.T.C.	0·164	0·134	0·176	0·206	0·070	0·091	0·054	0·105	47	49	64	49
0·14 per cent. P.T.C.	0·234	0·204	0·200	0·220	0·077	0·083	0·086	0·087	52	49	51	55
0·16 per cent. P.T.C.	0·195	0·216	0·178	0·206	0·094	0·089	0·103	0·111	47	56	50	55
$++$												
Control	0·075	0·089	0·081	0·080	0·102	0·076	0·088	0·088	61	50	62	59
0·02 per cent. P.T.C.	0·127	0·091	0·144	0·152	0·097	0·079	0·091	0·092	46	39	57	50
0·04 per cent. P.T.C.	0·116	0·103	0·202	0·169	0·097	0·088	0·087	0·079	60	65	52	53

In all cases, coefficients of variation increased with P.T.C. concentration. Flies differed in size between sexes (fig. 1), but for a given treatment the coefficients of variation differed very little, so suggesting that the coefficient of variation assesses variability tolerably well, and is independent of the mean.

There was little difference in variability due to the levels of competition. Assuming that each coefficient of variation is equally accurate, which for $n>30$ is a fair approximation, an analysis of variance of the coefficients of variation can be done for each genotype to test possible effects of sex, competition and P.T.C. concentration. Multiplying the coefficients of variation by 1000 for convenience, we get the analyses of variance of the coefficients of variation as presented in table 2. For all genotypes, variation in P.T.C. concentration

is significant, and for the homozygotes, there is a suggestion of a competition effect, such that at the high level of competition, the coefficients of variation are slightly higher than at the low level.

TABLE 2

Analyses of variance of the coefficients of variation of fly weight presented in table 1

	d.f.	M.S.	P.
e″e″			
Levels of P.T.C.	2	3878·58	<0·05
Competition	1	1656·75	<0·20
Sex	1	6·75	>0·20
Competition × Sex	1	140·08	>0·20
Error	6	476·36	...
e″+			
Levels of P.T.C.	6	9543·29	<0·001
Competition	1	488·89	>0·20
Sex	1	54·32	>0·20
Competition × Sex	1	308·89	>0·20
Error	18	387·29	...
++			
Levels of P.T.C.	2	4655·08	<0·05
Competition	1	4294·08	<0·05
Sex	1	310·08	>0·20
Competition × Sex	1	6·75	>0·20
Error	6	682·64	...

If, as before, we regard the coefficients of variation as equally accurate, *i.e.* contributing equal information in the statistical sense, then we can add the 4 coefficients for each level of P.T.C., and do a regression analysis of these summed coefficients on P.T.C. concentration for each genotype. The regression coefficients obtained, with

TABLE 3

Significance of regression of coefficients of variation of fly weight on P.T.C. concentration

Genotype	Regression coefficient	Probability
e″e″	1·25	<0·05
e″+	0·31	<0·001
++	1·32	<0·20

levels of significance, are given in table 3, and indicate that as P.T.C. concentration was increased, the coefficients of variation increase in parallel.

All cultures were classified daily until emergence had finished to obtain total emergence figures. To compare these, percentage emergence is given in table 4 for the 3 genotypes. This is the easiest method

of comparison, since variable numbers of replicates were used during the course of the experiment. As might be expected, percentage emergence fell as P.T.C. concentration was increased. There was little difference in levels of competition. Parsons and Kroman (1960) found that more flies emerged at the high level than at the low level on 0·04 per cent. P.T.C. This effect does not occur consistently in the results reported here, so the phenomenon, although statistically significant in Parsons' and Kroman's (1960) data, apparently depends

TABLE 4

Percentage emergence

Larvæ per replicate	*e″e″*	
	25	100
Control	57	50·5
0·01 per cent. P.T.C.	72·5	47·5
0·02 per cent. P.T.C.	61·5	46·5
0·03 per cent. P.T.C.	9·33	28·67
0·04 per cent. P.T.C.	6	3·75

Larvæ per replicate	*e″*+		++	
	25	100	25	100
Control	90	76·5	91·5	83
0·02 per cent. P.T.C.	82·67	75	97·6	82·5
0·04 per cent. P.T.C.	69	75	90·67	52
0·08 per cent. P.T.C.	44·44	51·5	17·33	15·67
0·10 per cent. P.T.C.	47·25	38	29·16	25
0·14 per cent. P.T.C.	33·33	31·33	21·68	15
0·16 per cent. P.T.C.	31·5	29	12·75	19·25
0·20 per cent. P.T.C.	11·25	8·75	10·5	4·5
0·24 per cent. P.T.C.	12·25	5	4·5	0·5
0·30 per cent. P.T.C.	3·75	3	1·5	2·75
0·34 per cent. P.T.C.	1	1·25	0	0

considerably on the precise culture conditions. At lower concentrations (0·02 and 0·04 per cent. P.T.C.), there was no great difference in emergence between ++ and *e″*+, but at concentrations above 0·08 per cent. P.T.C., *e″*+ became heterotic as found by Parsons and Kroman (1960).

In table 5, means and coefficients of variation of emergence time (the time taken for newly hatched larvæ to emerge as adults) are given. Both increased as P.T.C. concentration increased, and in all cases they were larger at the high level of competition than at the low level. Parsons and Kroman (1960) reported similar results. Hence, correlated with decreasing fly weight and increasing variability of fly weight is a prolongation of emergence time.

4. CHAETA NUMBER AND P.T.C. CONCENTRATION

In fig. 2, chaeta number is plotted against P.T.C. concentration for each sex, level of competition and genotype. The graphs show (1) females had more chaetæ than males for a given level of competition and P.T.C. concentration, (2) flies at the high level of competition had fewer chaetæ than those grown at the low level for a given sex on a given concentration of P.T.C., and (3) chaeta number decreased as P.T.C. concentration was increased. The greatest decrease of chaeta number occurred on low concentrations of P.T.C.

TABLE 5

Means and coefficients of variation of emergence time

Larvæ per replicate	Emergence time (days)		Coefficient of variation of emergence time	
	25	100	25	100
e″				
Control	7·63	8·01	0·066	0·093
0·01 per cent. P.T.C.	7·75	8·05	0·067	0·109
0·02 per cent. P.T.C.	8·64	10·23	0·061	0·109
e″+				
Control	7·02	7·42	0·018	0·067
0·02 per cent. P.T.C.	7·06	7·69	0·039	0·077
0·04 per cent. P.T.C.	7·21	8·17	0·079	0·110
0·08 per cent. P.T.C.	8·91	9·79	0·132	0·124
0·10 per cent. P.T.C.	8·47	10·29	0·108	0·147
0·14 per cent. P.T.C.	9·45	11·35	0·083	0·198
0·16 per cent. P.T.C.	9·56	11·67	0·084	0·199
++				
Control	7·11	7·52	0·044	0·086
0·02 per cent. P.T.C.	7·24	9·02	0·075	0·107
0·04 per cent. P.T.C.	7·98	10·57	0·097	0·136

Fly weight and chaeta number therefore react similarly to the various stresses. This suggests that the two variables are correlated. This possibility is discussed in section 5.

In table 1, coefficients of variation of chaeta number are given, but unlike fly weight no trend is obvious. Furthermore, the analyses of variance of the coefficients of variation showed no trends.

We can also study asymmetry of chaeta number between the 2 sides of the fly. Asymmetry has been measured as the summed absolute differences A between the 2 sides of a set of flies, divided by their total chaeta number T by Thoday (1955, 1958) and Beardmore (1960). This measure of asymmetry has been recently criticised by Reeve (1960), who maintains that there are always 3 macro-chaetæ on each side of the fly, hence we should divide by the total chaeta number minus 6. However, it is not possible in all flies to find 3 macro-chaetæ;

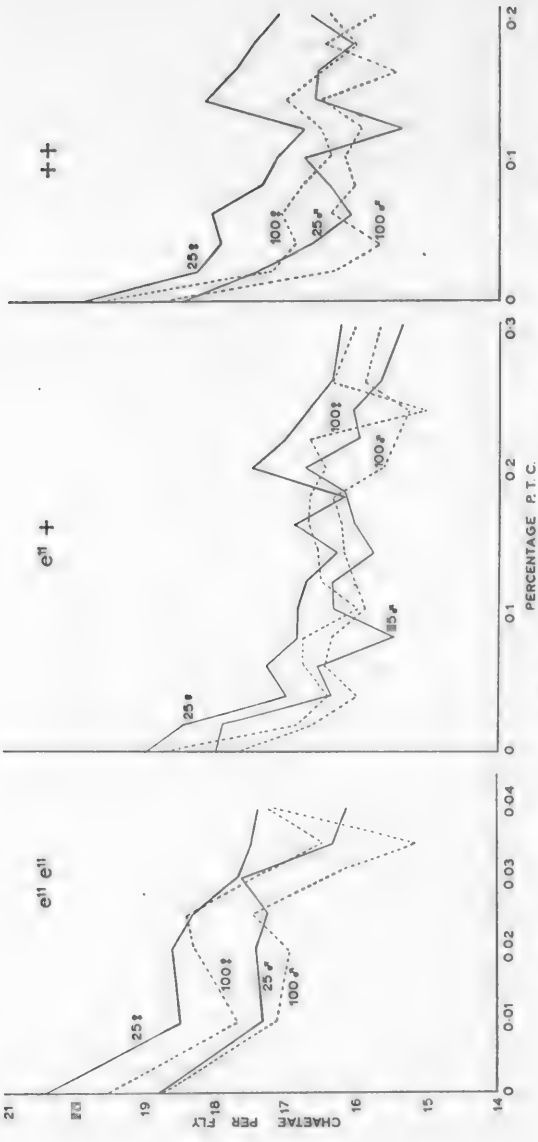

FIG. 2.—Mean chaeta number at different concentrations of P.T.C. for the genotypes $e^{11}e^{11}$, $e^{11}+$, and $++$.

in small flies there are often 2, and sometimes it is hard to distinguish macro- and micro-chaetæ. For this reason, it does not appear justifiable to change from the measure $\frac{A}{T}$ postulated above. Such values were estimated, and for convenience multiplied by 1000 (table 1). If all asymmetry values are assumed to be equally informative, analyses of variance can be done on them. No significant effect of genotype, sex, competition, or P.T.C. concentration was apparent on asymmetry. Thus the stress of P.T.C. which increased fly weight variability, did not increase chaeta number variability or asymmetry, even though there appears to be a correlation between chaeta number and fly weight. This conclusion is perhaps a little surprising, but may be resolvable if we can determine more precisely when chaeta numbers and positions are determined in the developing fly, and the variability of fly size at this stage.

5. CHAETA NUMBER AND FLY WEIGHT

As a preliminary, mean chaeta number was plotted against fly weight for each sex and level of competition for each concentration of P.T.C., provided that the mean was based on more than 5 observations. In fig. 3, the resultant scatter diagrams are given for the 3 genotypes. It is obvious that chaeta number and fly weight are correlated as suggested earlier from figs. 1 and 2. Fig. 3 shows that this correlation appears to be linear, thus chaeta number is proportional to fly weight.

To confirm this, a linear regression analysis of chaeta number on fly weight must be done. Such regression coefficients, which are all highly significant ($P < 0{\cdot}001$), are given for various contrasts in table 6. Comparing the total data for the 3 genotypes, we see that the *e″*+ coefficient is less than the ++ and *e″e″* coefficients. In fact, a test of significance (table 6) reveals that this difference is significant. Thus chaeta number decreased relatively less rapidly with decreasing weight in the heterozygotes than in the homozygotes. We have seen that the heterozygotes survive better at higher concentrations of P.T.C. (table 4). If sterno-pleural chaeta number is of adaptive value, as is certainly the case, then it is reasonable that there is a relationship between greater survival ability of *e″*+, and a smaller rate of decrease of chaeta number with decreasing fly weight, than occurred in the homozygotes. The heterozygotes may therefore have somewhat better developmental stability than the homozygotes.

We can also test for the effects of sex and competition within each genotype. If all the regression coefficients given in table 6 (*i*) (*b*) are assumed to be equally accurate, *i.e.* contribute equal information, a simple *t* test will test the difference between sexes and levels of competition. The assumption of equal information is reasonable, since the number of degrees of freedom on which the coefficients in

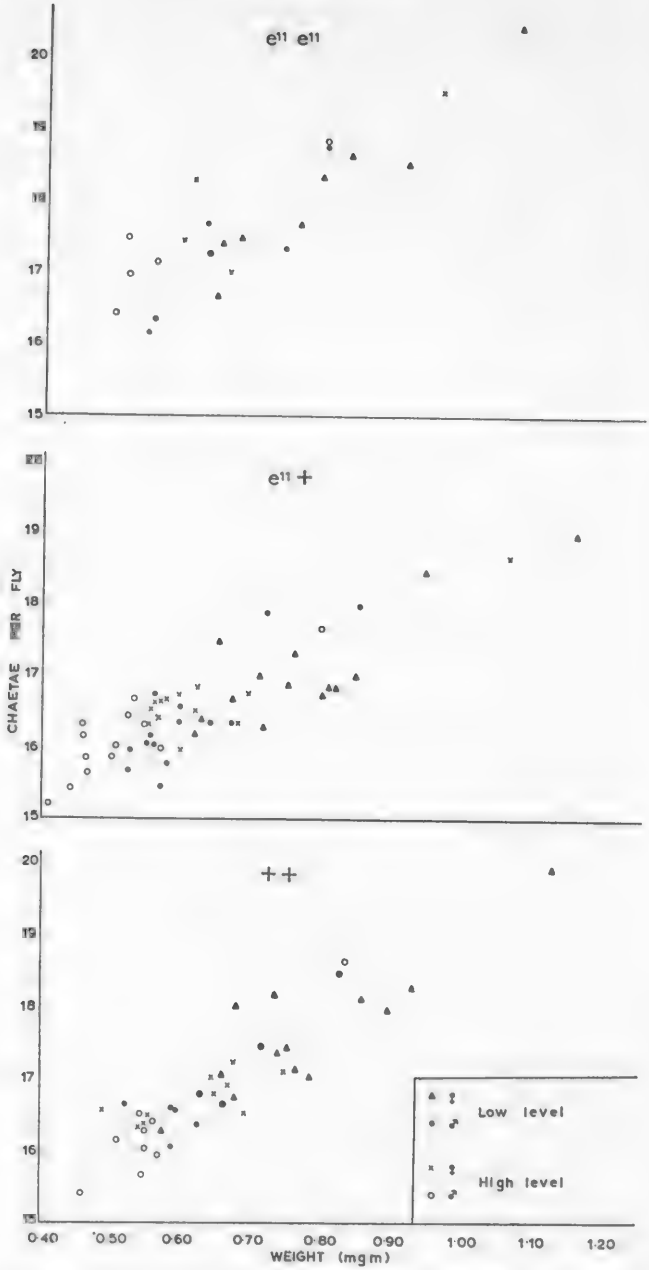

FIG. 3.—The relation between fly weight and chaeta number for the genotypes $e^{11}e^{11}$, $e^{11}+$ and $++$.

table 6 (*1*) (*b*) are based do not vary much within genotypes. Mean values of the regression coefficients are given in table 6 (*1*) (*c*) for sexes and levels of competition.

The mean values show that for all genotypes and levels of competition, the male regression lines are steeper than the female regression lines, and these differences are significant (table 6 (*2*)). An interpretation of this sex difference is a little difficult to find, but it may be a result of variability of female flies induced by egg formation.

TABLE 6

(1) *Regression coefficients of chaeta number on fly weight (mgm.)*

	e″e″	*e″+*	*++*
(*a*) Total data	0·053	0·042	0·057
(*b*) Low level of competition			
Females	0·050	0·043	0·052
Males	0·066	0·060	0·059
High level of competition			
Females	0·042	0·040	0·053
Males	0·069	0·053	0·073
(*c*) Totals (competition)			
Low	0·058	0·051	0·056
High	0·055	0·046	0·063
Totals (sexes)			
Females	0·046	0·041	0·053
Males	0·067	0·056	0·066

All regression coefficients are significantly $>0 (P<<0{\cdot}001)$.

(2) t *tests on the regression coefficients in table 6* (*1*) c

	e″e″	*e″+*	*++*
Low *v.* High level of competition	$0{\cdot}7<P<0{\cdot}8$	$0{\cdot}2<P<0{\cdot}3$	$0{\cdot}1<P<0{\cdot}2$
Females *v.* Males	$P<0{\cdot}02$	$P<0{\cdot}001$	$P<0{\cdot}01$

Total data	*e″e″* v. *e″+*	$P<0{\cdot}02$
	e″e″ v. *++*	$0{\cdot}2<P<0{\cdot}3$
	e″+ v. *++*	$P<0{\cdot}001$.

The level of competition has no effect on the slope of the regression lines. Presumably, as shown in figs. 1 and 2, the high level of competition leads to smaller flies, with proportionately fewer chaetæ, but the rate of decrease of fly weight with chaeta number does not vary between levels of competition. It appears therefore that a competition effect found by Gibson (unpublished) is related to fly size. He found that flies grown in 4-inch vials had fewer sterno-pleural chaetæ than those grown in half-pint milk bottles, presumably because competition is more intense in vials so giving smaller flies. Furthermore, Rasmuson (1952) found that flies had fewer abdominal and sterno-pleural chaetæ at a high level of competition.

6. FLY WEIGHT, TEMPERATURE AND CHAETA NUMBER

Firstly, we will consider the effect of temperature on the control medium (table 7). The following conclusions are apparent; (1) fly weight was reduced by increased temperature, (2) chaeta number was reduced by increased temperature as found in previous experiments (see Rasmuson, 1952, and Thoday, 1958), (3) fly weight and chaeta number were lower at the high level of competition at a given temperature than at the low level, (4) at the low level of competition, coefficients of variation of fly weight did not differ appreciably between temperatures, but at the high level, the variability of fly weight was

TABLE 7

Fly weight and chaeta numbers at 25° C. and 30° C.

	Fly weight (mgm. × 100)		Fly weight coefficient of variation		Chaeta no.		Chaeta no. coefficient of variation		Chaeta no. asymmetry $\left(\frac{A}{T} \times 1000\right)$	
25° C.	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control (Low) *	111·38	84·98	0·077	0·091	19·90	19·16	0·075	0·086	60	60
Control (High) †	88·38	66·55	0·111	0·090	17·87	17·17	0·077	0·079	51	59
0·02 per cent. P.T.C.†	60·15	49·73	0·189	0·220	16·89	16·25	0·086	0·083	37	59
0·04 per cent. P.T.C.†	54·58	45·44	0·206	0·196	17·05	16·02	0·092	0·068	53	61
30° C.										
Control (Low) *	107·61	80·07	0·074	0·087	18·98	18·07	0·091	0·092	72	45
Control (High) †	79·97	60·62	0·127	0·128	16·82	16·13	0·093	0·091	69	53
0·02 per cent. P.T.C.†	65·81	54·53	0·206	0·176	16·86	15·49	0·092	0·091	62	56
0·04 per cent. P.T.C.†	58·44	49·27	0·224	0·188	16·44	15·27	0·092	0·078	68	49

* Four replicates of 25 larvæ.
† One replicate of 150 larvæ.

greater at 30° C. than at 25° C., and (5) at 25° C. the coefficients of variation of chaeta number were lower than at 30° C.

Thus at 30° C. the flies were smaller than at 25° C., and correlated with this was a reduction in chaeta number. Variations in sternopleural chaeta number reported by other authors as due to temperature, and level of competition, therefore appear to depend at least partly on fly size. Preliminary results reported by Gibson, Parsons and Spickett (1961) confirm this.

Fly weight variability appears little affected by temperature, which is in contrast with P.T.C., where even a very low concentration of P.T.C. was effective in increasing fly weight variability. The combination of high temperature and high competition increased variability somewhat, but not to the same extent as P.T.C. in the medium. A temperature of 30° C. is a severe stress, for at 31° C. it is unlikely that more than a few flies would survive. Chaeta number variability was, however, considerably greater at 30° C. than at 25° C., whereas

P.T.C. has been shown not to affect chaeta number variability appreciably.

Data were also collected at the high level of competition on P.T.C., for each temperature. Fig. 4 shows that as expected P.T.C. reduced fly weight, but less for the 30° C. data than for the 25° C. data. Such an interaction, if significant, is a little difficult to explain, but appears to be some sort of complex genotype × environment interaction. Chaeta number was reduced by the addition of P.T.C. as expected, but the trends are not so obvious as for fly weight.

As expected, fly weight variability was increased considerably as a result of P.T.C. treatment. To find out the major cause of the

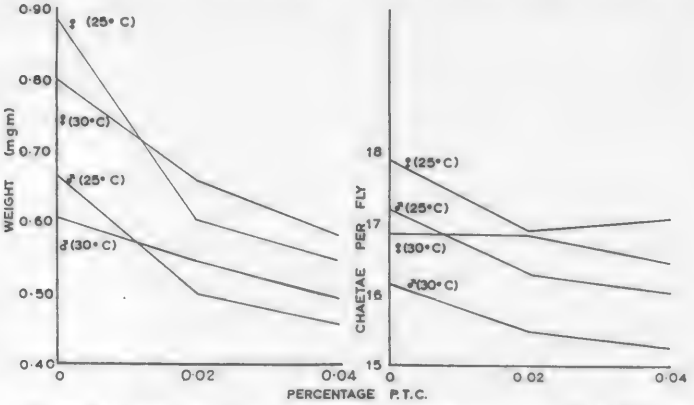

Fig. 4.—Mean fly weight and chaeta number at various concentrations of P.T.C. for the two temperatures 25° C. and 30° C.

variability of the coefficients of variation, we can do an analysis of variance to test the effect of the different treatments and sexes (table 8), assuming, as before, that the coefficients of variation are equally informative. The data collected on 0·04 per cent. P.T.C. were ignored, since very few flies emerged at 30° C. The competition and P.T.C. component is significant, showing that the variability in these data differs with level of competition and P.T.C. treatment. Although, as pointed out, the data appear more variable at 30° C. than 25° C., this increase in variability is not significant in an analysis of variance.

A high level of competition with P.T.C. present increased emergence time and its variability (table 9) as expected from the results already presented (table 5). At 30° C. emergence time was less than at 25° C., especially on 0·02 per cent. P.T.C. Thus correlated with a smaller fly at 30° C., and fewer chaetæ was a reduced development time.

Chaeta number variability was not affected by the presence of P.T.C., in agreement with previous results (table 1). In table 8 (*b*) an analysis of variance of the coefficients of variation confirms that at

TABLE 8

Analyses of variance of the coefficients of variation of fly weight and chaeta number, and of chaeta asymmetry (from data presented in table 7)

(a) Analysis of variance of the coefficients of variation of fly weight			
		M.S.	P
Competition and P.T.C. . . .	2	14241·59	P<0·001
Sex	1	5·33	P>0·20
Temperature	1	33·33	P>0·20
Temperature × Sex	1	133·33	P>0·20
Error	6	347·58	...
(b) Analysis of variance of the coefficients of variation of chaeta number			
Competition and P.T.C. . . .	2	9·33	P>0·20
Sex	1	5·33	P>0·20
Temperature	1	341·33	P<0·01
Temperature × Sex	1	12·00	P>0·20
Error	6	12·89	...
(c) Analysis of variance of chaeta asymmetry (measured as $A/T \times 1000$)			
Competition and P.T.C. . . .	2	33·08	P>0·20
Sex	1	56·33	P>0·20
Temperature	1	147·00	P<0·20
Temperature × Sex	1	385·33	P<0·10
Error	6	64·97	...

TABLE 9

Emergence time in days and its variability at 25° C. and 30° C.

	25 C. mean	Coefficient of variation	30 C. mean	Coefficient of variation	Significance of difference between means
Control (low) . .	7·20	0·059	6·86	0·056	P<0·001
Control (high) .	9·07	0·098	8·77	0·074	P<0·01
0·02 per cent. P.T.C.	10·58	0·173	9·47	0·108	P<0·001

30° C. chaeta number variability was greater than at 25° C. At 30° C. the mean coefficient of variation of chaeta number was 0·092 and at 25° C. it was 0·081.

Finally, chaeta asymmetry was calculated as $\frac{A}{T} \times 1000$. At 30° C. the mean value of $\frac{A}{T} \times 1000 = 59{\cdot}8$, and at 25° C. it came to 52·8,

assuming all asymmetry values to be equally informative (omitting the data for 0·04 per cent. P.T.C.). The analysis of variance of the asymmetry values (table 8*b*) is suggestive of a temperature effect, but more data are needed to confirm this although greater asymmetry at 30° C. agrees with Thoday's (1955 and 1958) results. There is a suggestion of a temperature × sex interaction, which if valid, is a little difficult to interpret.

Thus, at 30° C., flies deteriorate in developmental homeostasis as a result of the environmental change; accidents of development being more frequent at this temperature. It was noted, too, that out of 315 flies classified at 30° C., 10 had crumpled or curled wings, whereas at 25° C. out of 451 classified, none was affected. One fly at 30° C. was exceptional in having 24 sterno-pleural chaetæ on the left side and 9 on the right side. This fly was so grossly abnormal that it was omitted from the main analysis. These abnormalities merely confirm that at 30° C. developmental homeostasis breaks down fairly generally.

7. DISCUSSION

(i) *Variability of fly weight and chaeta number*

In this paper we are studying the effect of three environmental stresses, namely P.T.C., which is known to reduce fly size, temperature which is known to affect chaeta number, and competition. The results indicate a fairly direct correlation between fly size as measured by weight, and chaeta number for these three stresses.

The environmental stresses, P.T.C. and temperature, differ in nature. P.T.C. reduced fly weight and increased its variability. Emergence time was delayed by P.T.C., and its variability increased. P.T.C., however, had little effect on chaeta number variability and asymmetry. It therefore has little effect on the developmental stability of flies, and constitutes a stress affecting fly weight, but not the variability of the morphological structure of the fly.

Ebony larvæ die on lower concentrations of P.T.C. than wild-type larvæ (Kroman and Parsons, 1960), since the tyrosinase activity of ebony larvæ is lower (Ohnishi, 1954). Correlated with this is an increase of the size of the nuclei of the corpus allatum portion of Weismann's ring in ebony larvæ (Wolsky and Kalicki, 1959). Perhaps the somewhat longer development time of ebony larvæ (table 5) is connected with some sort of variation in juvenile hormone synthesis from the larger, and presumably more polytenic, nuclei of ebony larvæ, and this leads, as suggested by Wolsky and Kalicki (1959) to an eventual inhibition of tyrosinase activity. P.T.C., by inhibiting tyrosinase, may have the effect of delaying pupation by a complex interaction with the juvenile, or related, hormones. It is unlikely that such a mechanism would also increase chaeta number variability and asymmetry appreciably, whereas it seems reasonable that fly weight and emergence time are much more susceptible to P.T.C., since these are the components of fitness more directly under stress.

Temperature, however, increased chaeta number variability and asymmetry at 30° C. because of poorer developmental stability of the morphological structure of the fly, whereas fly weight and emergence time variability were little affected. In this case, the component of fitness which is reacting most to the stress by increasing its variability is chaeta number. Furthermore, other abnormalities were common, such as crumpled and curled wings. Although fly weight was reduced slightly, this was obviously not affected critically, and presumably at temperatures a little above 30° C., death would occur because of general poor homeostasis of the morphological structure of the fly.

P.T.C. specifically affects tyrosinase production and is therefore a much more "specific" environmental stress than temperature which probably affects many developmental pathways differentially so that various interactions would occur at different temperatures. Temperature is therefore a "general" environmental stress.

TABLE 10

A comparison of flies at 17° C. and 25° C. (150 larvæ per replicate)

	Weight (mgm. × 100)		Chaeta no.		Emergence time (days)	
	♀	♂	♀	♂	♀	♂
17° C. . .	98·57	81·56	19·87	18·95	16·96	17·05
25° C. . .	95·83	72·63	18·85	17·81	8·69	8·57

At 30° C. flies were smaller with fewer chaetæ and developed more rapidly than at 25° C., hence, at lower temperatures we would expect larger flies with more chaetæ. Observations by Rasmuson (1952), Thoday (1958), Beardmore (1960) and Plunkett (1926) show that more chaetæ are present at lower temperatures, and a small trial at about 17° C. recently carried out (table 10) confirmed that at lower temperatures an increase in fly weight was correlated with an increase in chaeta number and a longer emergence time (see also Gibson, Parsons and Spickett, 1961). Plunkett (1926) found that the chaeta number of flies was decreased progressively by growing larvæ for increasing periods at a high temperature after transferring from a lower temperature. Hence, no specific time during larval development is involved. We are dealing with a continuous process whereby fly size and chaeta number depend on the time of exposure to various temperatures. Auerbach (1936) showed that the two sterno-pleural discs separate during embryonic development; consequently the size of the discs will be affected by larval size, so influencing the size of the sterno-pleural plate and chaeta number.

On these arguments, in a line selected for high chaeta number, flies would be larger, and perhaps take longer to develop. It seems

reasonable that as selection proceeds interactions would occur, and the regression of chaeta number on fly size would become non-linear, such that chaeta number would increase relatively more than fly size since chaeta number is being selected directly. Preliminary results confirming this (Gibson, Parsons and Spickett, 1961) indicate that fly size as measured by weight and by the surface area of the sterno-pleural plate, is increased after selection for sterno-pleural chaeta number; but the increase in fly size is less than would be expected on the basis of the regressions presented in this paper. It seems worth while recording fly size and development time as concomitant observations during the progress of an experiment in which chaeta number is being selected. Non-linearity would be expected in the phenomenon of accelerated response to selection (Sismanidis, 1942; Mather and Harrison, 1949;

TABLE 11

	++		e″+	
	Chaetæ	Weight (mgm.× 100)	Chaetæ	Weight (mgm.× 100)
Control (25 larvæ per replicate)	18·42	82·13	17·99	84·88
0·16 per cent. P.T.C. (100 larvæ per replicate)	15·41	45·95	16·17	45·43
Per cent. reduction .	16·34	44·05	10·12	46·48

Thoday and Boam, 1961), since this is due to the occurrence of recombinants for high chaeta number between interacting polygenes. The recombinants are then favoured by selection, and so chaeta number would rise rapidly, whereas fly size would hardly be expected to be affected so radically.

(ii) *Chaeta number and fly size*

Reeve (1960) reported that a reduction of 23 per cent. in thoracic area was accompanied by a reduction in sterno-pleural chaeta number of 18·5 per cent. in the mid-parent between two Pacific inbred lines and 13·1 per cent. in the F_1. Comparing ++ and e″+ we get, for example, the results shown in table 11.

Hence the per cent. weight reduction is greater than the per cent. chaeta reduction, and on the basis of Reeve's data, it is also greater than the surface area reduction, since about the same chaeta reduction (< 20 per cent.) is accompanied by about 45 per cent. weight reduction and 23 per cent. thoracic area reduction. In small flies, therefore, chaetæ are more crowded than in large flies, and this occurs for sternite chaetæ (Robertson and Reeve, 1954). It seems legitimate to conclude

that chaeta number falls with decreasing surface area and volume but less rapidly than both.

Robertson and Reeve (1954) found that females had 20 per cent. more abdominal sternite chaetæ than males, and on the basis of the linear dimensions of the flies, they concluded that sternite chaeta density is the same in both sexes. The sex-difference for sterno-pleural chaetæ, however, appears to be less; the females having 5-10 per cent. more chaetæ than the males in the data presented in this paper. The presence of 3 macro-chaetæ on each side in most flies indicates a greater degree of differentiation in the sterno-pleural plate than in the sternites. The macro-chaetæ, although occasionally absent in smaller flies, vary less than the micro-chaetæ, perhaps because they are of greater adaptive significance. This may have the effect of making the sex-difference for sterno-pleural chaeta number less than for sternite chaeta number. In general, it may be expected that the more closely related to fitness a morphological structure is, the less variable it will be.

8. SUMMARY

1. Larvæ of three genotypes, ebony, wild-type and heterozygous ebony were grown on various concentrations of phenyl-thio-carbamide (P.T.C.) at two levels of competition, namely 25 and 100 larvæ per replicate. In general, as P.T.C. concentration increased, fly size decreased, and fly size variability increased. Emergence time and its variability both increased with increasing P.T.C. concentration. Sterno-pleural chaeta number decreased, but its variability was unaffected, which suggested that P.T.C. did not affect the developmental stability of the components of the fly, but only the size of the fly, perhaps by an interference with hormone production.

2. At the high level of competition, flies were smaller, had fewer chaetæ, and took longer to emerge than at the low level, but fly weight variance and chaeta number variance were not affected, although emergence time variance increased, no doubt in response to the additional stress involved.

3. The linear regression of fly weight on chaeta number was significant for all genotypes, but the regression line for the heterozygotes was significantly less steep than for the homozygotes, perhaps due to better homeostasis of the heterozygotes. A sex difference in regression lines was found and is perhaps due to the greater variability of females with developing eggs.

4. As the flies became smaller, the chaetæ became more crowded, even though there was a reduction in chaeta number. The percentage reduction of weight was greater than the percentage surface area reduction which was greater than the percentage chaeta number reduction, when comparing different fly sizes.

5. Flies grown at 30° C. were smaller, developed more rapidly, and had fewer chaetæ than those grown at 25° C. Fly weight, and

emergence time variability were not affected appreciably at 30° C., but sterno-pleural chaeta variability and asymmetry were increased, suggesting that temperature is a more "general" stress than P.T.C. for it affects the developmental stability of the components of the fly. The temperature data also indicate that fly size is in general correlated with chaeta number and emergence time. Hence, if in a selection experiment, chaeta number is being selected, it is plausible that there may be a partially correlated response of emergence time and fly size.

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THE DARWINIAN FITNESS OF POLYMORPHIC AND MONOMORPHIC POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA* AT 16° C.

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1. INTRODUCTION

NATURAL populations of many species of *Drosophila* show a chromosomal polymorphism which is due to inversions of block of genes. Over the range of environments common in the natural habitats, this polymorphism is adaptive and balanced; the heterokaryotypes possess an adaptive value superior to that of the homokaryotypes.

As shown by Dobzhansky (1948, 1957), Levine (1955) and Levene, Pavlovsky and Dobzhansky (1958), experimental populations of *Drosophila pseudoobscura* containing third chromosomes with AR and with CH gene arrangements are subject to strong selection, which at 25° C. conduces to an equilibrium of about 70 per cent AR and 30 per cent CH. Beardmore, Dobzhansky and Pavlovsky (1960) have made an attempt to compare the fitness of experimental populations of *Drosophila pseudoobscura* polymorphic and monomorphic for the above gene arrangements. They estimated certain parameters which were considered significant for evaluation of fitness. The results indicated that the polymorphic populations studied were superior to the monomorphic ones. However, since the experiments were carried out at 25°, it was not possible to decide whether the differences between the polymorphic and the monomorphic populations were due to a superiority of the heterozygotes or to a mutual facilitation between the carriers of the different karyotypes. It was conjectured that both factors were involved.

In order to throw more light on this question, we repeated the experiments with the same populations but kept them at 16° C., a temperature at which heterozygotes are known not to be superior to homozygotes. This is the scope of the present work.

2. MATERIAL AND TECHNIQUES

The three experimental populations used were studied by Beardmore *et al.* (1960) at 25° C. Population No. 180 was monomorphic for AR, No. 181 was polymorphic for AR and CH, No. 182 was monomorphic for CH. In August 1958, the three population cages were transferred from 25° to a constant temperature

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room at 16°. The populations were sampled between 9th September 1958 and 3rd March 1959. From time to time a cup with food in which pupæ were beginning to form was withdrawn from each cage, attached by adhesive tape to a glass "chimney" provided with a cotton stopper and when the adults began hatching, they were collected daily, etherised, counted and weighed individually on a misco quartz helix balance. Then they were preserved in alcohol. Details of the methods adopted and of the foundation stocks of the experimental populations, are given by Beardmore, Dobzhansky and Pavlovsky (1960).

Ten random samples were taken from each population for the purpose of measuring wing length. The number in each sample was fifty flies of each sex, or the total emergence if this was less than fifty flies. Both wings were removed from each fly and mounted under cover-glasses, right and left wings forming separate rows.

The measurements were made using the Promar (Seibert-Wetzlar) projecting microscope of the Zoology Department, University of Padua, Italy. This permitted easy measurement with the aid of a ruler of the image of the wing projected onto a sheet of white paper. The wing length was measured from a point near the posterior end of the humeral crossvein to the tip of the wing near the end of the third vein.

3. RESULTS

(i) *Numbers of flies produced*

Table 1 shows the means, variances and variation coefficients of the numbers of the flies which hatched from the cups taken from the

TABLE 1

Number of flies hatching per cup in the three populations

Population	Sex	Mean	Variance	Variation coefficient	Cups examined
180, AR	Female	258·00±36·2	18,651	52·7	14
181, AR+CH . .	Female	304·78±44·8	28,345	55·1	14
182, CH . . .	Female	270·14±29·6	12,249	41·5	14
180, AR	Male	202·28±28·2	11,251	52·4	14
181, AR+CH . .	Male	268·28±34·9	17,287	48·8	14
182, CH . . .	Male	234·07±29·3	12,036	47·0	14

three population cages. For the reasons stated by Beardmore, Dobzhansky and Pavlovsky (1960), the number of the adults that hatch is an indication of the ability of the different genotypes in a given population to exploit successfully the available environmental opportunities.

The comparison of the polymorphic (181) and the two monomorphic populations (180 and 182), which have been sampled at the same time, indicates that the number of flies hatching per cup is higher in the polymorphic population in both sexes. However, these differences are not statistically significant. The variances and the coefficients of variation indicate that the variability in the polymorphic population is not lower than in the monomorphic ones. It seems that population 182 (CH) is least variable, but apparently the differences are not significant.

(ii) *Weight and biomass*

The flies hatching in each of fourteen cups in the three populations studied were weighed individually. The mean weights of the flies of both sexes are given in tables 2 and 3, together with the number

TABLE 2

Weight (in mg.) of flies in the three populations. Females

No. 180, AR			No. 181, AR+CH			No. 182, CH		
Mean	s^2	Flies	Mean	s^2	Flies	Mean	s^2	Flies
0·754	0·055	233	0·800	0·020	243	0·720	0·032	226
0·800	0·037	168	0·636	0·029	293	0·737	0·020	234
0·622	0·021	436	0·577	0·033	561	0·675	0·026	329
0·700	0·042	251	0·552	0·026	528	0·685	0·028	389
0·760	0·047	498	0·657	0·027	571	0·658	0·036	462
0·696	0·031	290	0·652	0·028	490	0·723	0·043	294
0·591	0·036	467	0·593	0·026	386	0·607	0·018	440
0·924	0·012	114	1·165	0·023	124	0·767	0·030	340
0·974	0·037	67	0·952	0·020	204	0·751	0·036	257
1·058	0·053	132	0·996	0·057	122	0·958	0·039	155
0·854	0·084	306	0·946	0·090	161	1·133	0·020	108
0·620	0·032	288	0·689	0·055	186	0·784	0·070	182
1·128	0·059	105	0·978	0·053	155	1·156	0·045	135
0·669	0·084	257	0·688	0·024	243	0·536	0·022	231

TABLE 3

Weight (in mg.) of flies in the three populations. Males

No. 180, AR			No. 181, AR+CH			No. 182, CH		
Mean	s^2	Flies	Mean	s^2	Flies	Mean	s^2	Flies
0·693	0·047	175	0·732	0·019	233	0·706	0·026	181
0·754	0·027	154	0·603	0·020	275	0·689	0·021	160
0·583	0·014	413	0·531	0·011	497	0·625	0·042	318
0·661	0·081	125	0·516	0·018	460	0·602	0·023	311
0·676	0·034	387	0·573	0·020	442	0·605	0·022	482
0·616	0·030	224	0·603	0·018	435	0·667	0·024	285
0·549	0·029	223	0·547	0·026	218	0·539	0·012	318
0·817	0·013	125	1·085	0·014	148	0·718	0·020	313
0·852	0·028	46	0·893	0·014	173	0·719	0·025	261
0·943	0·026	117	0·854	0·045	127	0·869	0·018	130
0·830	0·076	230	0·883	0·063	154	1·024	0·014	86
0·575	0·032	292	0·635	0·042	185	0·700	0·033	140
0·978	0·046	98	0·896	0·038	171	1·020	0·026	142
0·609	0·043	223	0·647	0·017	238	0·526	0·027	150

of flies in each cup and the variances of the individual weights (s^2). The intra-cup variances, *i.e.* the average variances of the individual flies emerging in the same cup, and the inter-cup variances, *i.e.* the

variances of " means ", for each population and sex, are given in table 4.

In contrast to what has been observed in the experiments of Beardmore *et al.*, in our experiments the variances, both intra-cup and inter-cup, do not differ significantly in the three populations. The same is shown by the coefficients of variation, which have been computed in order to eliminate the effects of the differences in the average weights. The coefficients of variation range for females from 13·0 to 34·1 in the polymorphic population AR+CH, from 11·9 to 43·2 in the population monomorphic for AR, and from 12·3 to 33·8 in the population monomorphic for CH. For males, they range from 10·9 to 32·3 in the polymorphic population, from 13·9 to 42·9 in the population AR, and from 11·6 to 32·6 in the monomorphic CH. Considering only the variances of individuals and the coefficients of variation,

TABLE 4

Mean weight (in mg.) of a fly, and intra-cup variances (of individuals) and inter-cup variances (of means), in the three populations

Population	Sex	Mean	Intra-cup variance	Inter-cup variance
180, AR . .	Female	0·797	0·045	0·020
181, AR+CH .	Female	0·777	0·036	0·038
182, CH . .	Female	0·778	0·033	0·033
180, AR . .	Male	0·724	0·037	0·020
181, AR+CH .	Male	0·714	0·026	0·031
182, CH . .	Male	0·715	0·024	0·024

the population 182 (CH) seems to be the least variable. This, however, is not confirmed by the variances of the means which are not in agreement with the intra-cup data. The absence of a regular trend in the three series of data (intra-cup variances, coefficients of variation, inter-cup variances) makes the differences observed not significant.

There is no significant difference between the weights of flies belonging to the polymorphic population and the weights of those belonging to the two monomorphic ones. However, there is a slight inverse correlation between the number of flies produced in a cup and their weights. Flies developing at 16° C. are somewhat lighter than those produced by the same populations at 25° C., from which hatch much smaller numbers of flies. There is nevertheless some indication (table 2) that, with equal population densities, the flies are slightly heavier in polymorphic than in monomorphic populations.

Let us now consider the total weights of the flies emerging in the different populations with the same amount of food (the amount of food contained in a cup). These values (table 5) correspond to the biomass, and are obtained by multiplying the numbers of the flies hatching in the different cups by their mean weights. The data show

that the biomass is greater in the polymorphic population (181); this is true also for females and males considered separately. The data reported in tables 1, 4 and 5 show that the polymorphic population produces more flies, which are not much lighter than those produced in smaller numbers in the monomorphic populations.

TABLE 5

Total weights (biomass) in mg. produced per cup in the three populations

Sex	Population		
	180, AR	181, AR+CH	182, CH
Females	189·41	212·85	196·75
Males	137·51	175·51	158·01
Total	326·92	388·36	354·76

Thus at a temperature of 16° C., just as at 25° C., the polymorphic population, 181, is more productive than the monomorphic ones, 180 and 182.

(iii) *Wing length*

Table 6 gives the mean wing lengths of the flies, together with the intra-cup and inter-cup variances. The coefficients of variation are shown in table 7. The data indicate no difference in wing length

TABLE 6

Mean wing length (in mm.) and intra-cup variance and inter-cup variance in the three populations

Population	Sex	Mean	Intra-cup variance	Inter-cup variance
180, AR . .	Female	2·309	0·024	0·017
181, AR+CH .	Female	2·298	0·019	0·026
182, CH . .	Female	2·223	0·020	0·010
180, AR . .	Male	2·116	0·022	0·010
181, AR+CH .	Male	2·118	0·011	0·017
182, CH . .	Male	2·069	0·014	0·007

between the polymorphic and monomorphic populations. However, the flies, females and males, in population 182 (CH) have significantly shorter wings than the flies in populations 180 (AR) and 181 (AR+CH). This may partly depend upon environmental factors, such as the degree of crowding in the population cages.

Comparison of the "individual" variances (intra-cup) of wing length shows that the polymorphic population is less variable than the monomorphic ones. In fact, the females in the population 181 have

the variance of 0·01921 and in the populations 180 and 182, 0·02447 and 0·01988; the males show a variance of 0·01148 in the polymorphic and 0·02224 and 0·01403 in the monomorphic populations. These differences in variability become even clearer in the coefficients of variation (table 7).

TABLE 7

Mean wing lengths. Coefficient of variation for individuals

Population	Sex	
	Females	Males
180, AR	6·60	6·91
181, AR+CH . . .	5·96	4·93
182, CH	6·23	5·52

The variances of means (inter-cup) on the other hand behave differently, being greater in the polymorphic population than in the monomorphic ones, as in the case of the inter-cup variances of the fly weights. Here again we can say that the disagreement between the two types of variances greatly reduces the significance to be attached to the differences observed.

(iv) *Asymmetry*

The data on wing asymmetry, *i.e.* the mean intra-fly difference between right and left wings, calculated for each population and reported on table 8, are more informative. They clearly show that flies

TABLE 8

Mean asymmetry of wings in microns in flies of different populations

Sex	Population		
	180, AR	181, AR+CH	182, CH
Females	11·505	9·671	10·235
Males	11·705	9·121	10·893

from the polymorphic population (181) have lower average values of wing asymmetry than flies from monomorphic populations (180 and 182). A statistical comparison of the means (table 9) shows that the differences are highly significant in three cases out of four. The only exception is in the case of the females of the comparison 182>181. A similar result was observed in the population of Beardmore, Dobzhansky and Pavlovsky (1960) at 25° C. The same explanation can be given

in our case, namely that the flies from population 182 have shorter wings than those from population 181 (see table 6) and the "asymmetry value is therefore correspondingly lower than it would have been had the mean wing length of flies from 182 been more nearly equal to that of the 181 flies". As pointed out by Thoday (1958) the degree of asymmetry of bilaterally paired structures appears to depend in part on the absolute size of the structures themselves. We have converted the absolute asymmetry values into relative values, dividing them by the wing lengths. On doing so, the difference in wing asymmetry between the females of the populations 182 and 181 becomes

TABLE 9

Comparisons of mean values of wing asymmetry in the three populations

Sex	Populations	t	P
Females	180>181	3·162	<0·01
	182>181	1·007	0·4-0·3
Males	180>181	4·379	<0·001
	182>181	3·055	<0·01

statistically significant. This clearly shows that, at 16° as at 25° C., developmental homeostatic mechanisms are more efficient in the polymorphic than in the monomorphic populations.

4. DISCUSSION

At the time when our experiments were carried out, the genetic composition of the three populations studied, 180, 181 and 182, was approximately the same as in the experiments by Beardmore *et al.* The two monomorphic populations, 180 and 182, consisted respectively of flies homozygous for the AR and the CH gene arrangement of the third chromosome, whereas the polymorphic population, 181, consisted of flies belonging to the three karyotypes AR/AR, CH/CH and AR/CH, with AR being about twice as frequent as CH and, consequently, with slightly fewer than half of the flies belonging to the heterokaryotype AR/CH. At 16° the three karyotypes appear to have approximately the same adaptive values, as shown by the absence of changes in the frequencies of the gene arrangements.

The data reported in the present paper indicate that the polymorphic population produces more and, with equal population densities, slightly heavier flies, and consequently a greater biomass, than the monomorphic populations. As far as the variability is concerned, the polymorphic and monomorphic populations show inconsistent differences. Beardmore *et al.*, working with the same populations kept at 25°, observed lower variances in the polymorphic than in the monomorphic populations.

The absence of clear-cut differences between the variabilities in

polymorphic and monomorphic populations may be a reflection of the fact that the temperature of 16° is a less demanding environment than 25°, as shown indirectly by the far greater numbers of flies surviving at the lower temperature.

It may be of some interest to note that the difference in biomass between 25° and 16° data is accounted for by a greater number of flies and not by larger flies, at 16°; in fact the mean weight of a fly at the lower temperature is lower than it is at the higher one, though the figures are, for many reasons (time of sampling, etc.), not strictly comparable.

The significance of the differences in wing asymmetry, found at 16° as well as at 25°, is perhaps due to the more precise method of wing measurement adopted in the present work. It clearly shows that flies belonging to the polymorphic population have more symmetrical wings. May this be taken as an indication of a higher homeostasis in the polymorphic population? Many facts suggest that asymmetries are often the result of an imperfect homeostasis. A higher degree of homeostasis, as indicated by the lower variability of certain quantitative traits, is a property of heterozygous genotypes as compared with the homozygous ones. Now observations made by Mather (1953) and Tebb and Thoday (1954) in *Drosophila melanogaster* show that the degree of asymmetry of certain traits such as the numbers of sternopleural bristles on the two sides of the body, is greater in homozygotes than in heterozygotes. Moreover, according to Beardmore, Dobzhansky and Pavlovsky (1960), " it is hardly an accident that heterozygous genotypes, at least those heterozygous for co-adapted gene complexes derived from the same population, show both low variance in traits concerned with fitness and low asymmetry ".

Thus, even disregarding, because of the lack of statistical significance, the ability to produce a greater number of flies and a greater biomass shown by the polymorphic population in our experiments, other results show that the polymorphic population is superior in fitness to populations monomorphic for AR or for CH.

The persistence of the advantage, though reduced, at approximately optimal environmental conditions, without any superior fitness of the heterozygotes, such as is observed at 25°, would indicate that the higher fitness of the polymorphic population is, at least in part, to be attributed to phenomena of mutual facilitation between different genotypes. This is in agreement with the view expressed in a previous work by Beardmore *et al.* (1960).

Experiments by Levene, Pavlovsky and Dobzhansky (1954, 1958), Spiess (1957) and Lewontin (1955) have shown that the viability of a genotype is a function of the other genotypes coexisting with it in the same medium. In our case, the two karyotypes, AR/AR and CH/CH, would actually benefit by living side by side and together with AR/CH. The ecological mechanism which underlies this peculiar interaction remains, however, to be clarified.

5. SUMMARY

The observations reported here concern three experimental populations of *Drosophila pseudoobscura* kept at 16° C. One of them was polymorphic for the AR and CH gene arrangements in the third chromosome, one was monomorphic for the AR, and one for the CH chromosome. At the temperature employed, the heterozygotes AR/CH are not heterotic, as they are at 25° C. Thus the composition of our populations was the same as it was at 25° C., the temperature at which the three populations had been kept previously, namely, 70-75 per cent AR and 25-30 per cent CH, with about half of the individuals belonging to the heterokaryotype AR/CH.

Samples were taken from each of the three populations, at more or less regular intervals, in order to estimate certain parameters which were considered significant for evaluation of fitness.

The results show that (*a*) the polymorphic population produces more, and with equal population densities, slightly heavier flies than do the monomorphic ones; (*b*) the polymorphic population produces also a greater biomass; (*c*) the mean length of the wings does not differ significantly in polymorphic and monomorphic populations; (*d*) the variabilities of the numbers, weights, and wing lengths in the flies produced in the three populations show no consistent differences; (*e*) the asymmetry of the wings is significantly greater in monomorphic populations than in the polymorphic one.

The conclusion, mainly based on points (*a*) and (*e*), is that the polymorphic population is superior in fitness to the monomorphic ones. The persistence of such advantage, though to a lesser degree, at 16° C. as well as at 25° C., favours the view that the superior fitness of the polymorphic population is due at least in part to mutual facilitation between the carriers of the different gene arrangements.

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VIABILITY EFFECTS AND RECOMBINATION DIFFERENCES IN A LINKAGE TEST WITH PALLID AND FIDGET IN THE HOUSE MOUSE

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1. INTRODUCTION

The recessive gene pallid (*pa*) (Roberts, 1931) causes a dilution of the coat colour, pink eyes and an abnormality of the labyrinth (Lyon, 1953). It has generally been found to reduce viability to some extent. Fidget (*fi*) is one of the "shaker" group of mutants. It is a recessive which was first described by Grüneberg (1943). Affected animals are generally smaller and weaker than normal mice in the early stages of their development. This results in a marked reduction in both their viability and fertility. The two genes are known to be situated in linkage group V (Roberts and Quisenberry, 1935; Carter and Grüneberg, 1950; Wallace, 1950).

The viability effects of *pa* and *fi* and the fact that they are linked make them well suited to an analysis of viability effects and interactions in a balanced linkage experiment, using the methods of analysis developed by Bodmer and Parsons (1959*a*, *b*) and Bodmer (1959). The only linkage test using both *pa* and *fi* is due to Carter (1951) and is based on intercrosses. This region of chromosome V is, however, one which has been subject to much investigation. Sex differences in recombination have been reported by Fisher and Landauer (1953), Wallace (1957) and Parsons (1958*a*), and an effect of age on recombination has been analysed by Fisher (1949). The purpose of this paper is to describe the results of a balanced backcross linkage test using these two factors *pa* and *fi*.

2. METHOD

It is difficult to breed reliably from fidget mice and Wallace (1957) was in fact the first person to use *fi* in backcross linkage tests. By a systematic programme of selection she was able to increase markedly their breeding performance. Combination of the *pa* and *fi* genes caused a further acute reduction in viability and fertility so that, to start with, pallid fidget mice were almost unobtainable and exceedingly weak. However, selection improved their performance so that it was possible to breed from both male and female pallid fidget mice. The stocks of pallid fidgets were maintained largely by coupling or single backcrosses. As was to be expected, it proved more difficult to obtain data from male heterozygotes, when the pallid fidget mice were the females, than from female heterozygotes with pallid fidget males. Adequately balanced data have thus only been obtained from the female coupling and repulsion heterozygotes $\left(\frac{pa\,fi}{+\,+}\text{and}\frac{pa+}{+fi}\right)$ and only a smaller amount of data was obtained from male coupling heterozygotes.

3. ANALYSIS OF THE DATA FROM FEMALE HETEROZYGOTES

The improvement of the pallid and fidget viabilities by selection indicates the presence of some genetic variance affecting these characters. This should be demonstrable by variations in the proportion of fidgets and pallids between matings. The heterogeneity χ^2s for the ratios of fidget to non-fidget and pallid to non-pallid are shown for both coupling and repulsion series in table 1.

There is in both series of matings highly significant heterogeneity in the ratio of fidgets to non-fidgets but no evidence for heterogeneity in the ratios of pallids to non-pallids. Some of this variation may be due to the fact that data from different matings will not be balanced for the age of the mother and that viabilities may change significantly with the age of the mother, as will be discussed below. However, although it has not been possible to allow specifically for this, there

TABLE 1

Tests for the heterogeneity between matings

	Fidget : non fidget	Pallid : non-pallid
Coupling .	$\chi^2_{21} = 38{\cdot}3403$ $P \approx 1$ per cent.	$\chi^2_{21} = 19{\cdot}4137$
Repulsion .	$\chi^2_{18} = 43{\cdot}7676$ $P < 0{\cdot}1$ per cent.	$\chi^2_{18} = 19{\cdot}5418$

seems to be no particular bias in the ages of parents which produced unusually high or low fidget ratios. Thus, there is at least suggestive evidence for some genetic control of the fidget viability. More significant evidence for such control with respect to fidget than to pallid is to be expected, as selection has been operating mainly to improve the fidget viability.

Truslove (1956) observed a similar heterogeneity in the proportion of fidgets in a series of backcrosses and intercrosses derived from outcrosses of Grüneberg's (1943) original fidget stock. She obtained a $\chi^2_3 = 7{\cdot}78$ for the heterogeneity of the backcrosses and a $\chi^2_4 = 8{\cdot}37$ for that of the intercrosses which together give a significance level of 2·5 per cent.

The combined data from repulsion and coupling matings are summarised in table 2.

Analyses of χ^2 were performed on the original data (Bodmer and Parsons, 1959*a*) and on the data after taking logarithms (Bodmer, 1959) and these are shown in table 3. As would be expected, the recombination component is by far the largest in both analyses. There is also some lack of orthogonal balance indicated by the significant "heterozygotes" component. The viability × heterozygote and viability × recombination components are significant only on the χ^2

analysis on the original data. Thus, although the 2-factor viability interaction is slightly inflated in the logarithmic analysis, a multiplicative model fits the data better than an additive model, as was predicted by Bodmer (1959) and verified in *Drosophila* by Parsons (1959). The usual 2×2 contingency χ^2_1 calculated from the "sums" square

TABLE 2

Combined data from coupling and repulsion female heterozygotes

	Non-recombinants		Recombinants	
Coupling . . .	+	221	*pa*	61
	fi pa	93	*fi*	59
Repulsion . . .	*pa*	145	+	76
	fi	125	*fi pa*	25

for detecting 2-factor viability interactions, is 0·02 and in this case agrees more closely with the result from the additive analysis. However, it may well be that a multiplicative model with some slight viability interaction gives a more accurate representation of the underlying situation than an additive model with no viability interaction but

TABLE 3

Chi-squared analysis of data from female heterozygotes

	d.f.	Original data χ^2	P in per cent.	Logarithmic data χ^2	P in per cent.
Latin square of sums					
Heterozygotes . . .	1	4·93	5-2·5	7·36	1-0·5
Recombination . . .	1	163·69	$<<<0·1$	201·05	$<<<0·1$
2-factor viability interaction .	1	0·78	...	3·31	10-5
Latin square of differences					
Viability × heterozygote .	1	4·32	5-2·5	1·65	...
Viability × recombination .	1	11·21	$<0·1$	0·21	...
Main viability effect of *pa* .	1	30·62	$<<0·1$	40·56	$<<0·1$
Main viability effect of *fi* .	1	50·19	$<<0·1$	58·63	$<<0·1$
	7				

with spurious viability × heterozygote and viability × recombination interactions.

The estimate of the recombination fraction using the product formula (Fisher, 1935 and later) is 27·4416 per cent. ± 1·585 per cent. This is somewhat higher than the value of 19 per cent. ± 3·6 per cent. obtained by Carter (1951). However, his estimate was based on intercross data and is thus likely to be severely disturbed by any viability differences. Following Bodmer and Parsons (1959*b*) it is possible to estimate

the viabilities of pallid and fidget assuming a multiplicative model, a procedure which is similar to the estimation of the recombination fraction using a product formula. The estimate of the fidget viability is 58·68 per cent. ±5·01 per cent. and of the pallid viability 64·39 per cent. ±5·50 per cent. As expected, both the fidget and pallid conditions cause severe reductions in viability. It is interesting to note that the viability estimates obtained in the usual way from the over-all proportions of fidgets and pallids are 75·03 per cent. ±3·42 per cent. and 80·50 per cent. ±3·46 per cent. respectively, which just about differ at a 5 per cent. significance level from the multiplicative estimates.

TABLE 4

Combined data from female heterozygotes tabulated according to the age of the maternal parent

Age in months	Total No.	No. of litters	Fidgets		Pallids		Recombinants	
			No.	%	No.	%	No.	%
2	115	21	44	38·3	37	32·2	37	32·2
3	84	19	45	53·6	35	41·7	26	30·9
4	106	17	45	42·5	45	42·5	26	24·5
5	98	18	35	35·7	37	37·8	28	28·6
6	108	17	39	36·1	52	48·2	37	34·3
7	73	15	31	42·5	35	47·9	17	23·3
8	92	19	32	34·8	36	39·1	22	23·9
9	42	10	12	27·5	18	41·2	10	22·5
10	38	10	10		15		8	
11	24	7	6		10		5	
12	8	3	1	18·4	3	28·6	4	20·4
·12	17	7	2		1		1	
	805	163	302	...	324	...	221	27·45

The good fit of the logarithmic χ^2 analysis makes it almost certain that the multiplicative estimates provide a more genuine measure of the fidget and pallid viabilities.

Perhaps the most interesting aspect of the data is revealed by an analysis of the effect of the age of the maternal parent on the viabilities and on the recombination fraction. A summary of the combined coupling and repulsion data subdivided according to the age of the female parent is given in table 4. It is immediately apparent that there is a marked reduction in fidget viability with increasing age of mother, a similar but less marked reduction in the pallid viability and in the recombination fraction. Although, as pointed out above, the proportion of fidgets and pallids may not be very satisfactory estimates of the viabilities, these proportions are the only practicable measures which can be used for a regression analysis. Following Fisher (1949) the proportions were first transformed by the angular transformation ($\sin^2 \Phi = \rho$), so that the variance attached to any value depends only

on the observed total. An analysis of variance can then be performed on the transformed angular values. For this purpose the data for ages 9 and 10 months were combined, as were the data for all ages greater than or equal to 11 months.

A χ^2_1 for linear regression was calculated from a straightforward weighted linear regression analysis as described by Fisher (1949). An approximate χ^2_1 for quadratic regression was calculated by giving equal weight to the observations from different ages and using orthrogonal polynomials in the usual way (Fisher and Yates, 1957). The theoretical variance of the angular values was then calculated from the harmonic mean of the observed totals for the different maternal ages. The residual χ^2 was obtained by subtraction. The resulting regression analysis is given in table 5.

TABLE 5

Analysis of regression of viabilities and recombination fraction with maternal age

		Fidget viability	Pallid viability	Recombination fraction
Linear regression . .	χ^2_1	13·77***	0·02	4·08*
Quadratic regression † .	χ^2_1	4·20*	8·10**	0·18
Residual	χ^2_6	6·00	3·01	4·47

*** P<0·1 per cent., ** 0·5 per cent.>P>0·1 per cent., * 5 per cent.>P>2·5 per cent.
† Approximate test giving equal weight to observations from different ages (see text for explanation).
All analyses were performed after applying the angular transformation.

The linear regression of recombination fraction with increasing maternal age is significant at the 5 per cent. level and there is no evidence for a quadratic component or any significant departure from linearity. Variation in recombination with maternal age is well known in *Drosophila* and was first described by Bridges (1915). According to Bridges (1929) there is an initial decrease in recombination with a minimum at 9-11 days but then further increases and decreases giving a W-shaped curve of variation. The only other significant downward trend of recombination with maternal age in mice is reported by Fisher (1949). Wallace (1957) points out that her data, Fisher's data and that quoted by Owen (1953) all give U-shaped curves for the first 9 months, but such variation could hardly be judged to be significant.

The linear regression of fidget viability with maternal age is highly significant. The quadratic regression is significant at the 5 per cent. level, but not too much weight must be placed on this as the calculated χ^2_1 is an approximate one. The pallid viability, however, shows no

significant linear regression but a highly significant quadratic regression whose significance is not likely to be materially affected by the approximations used. It is well known that average litter size decreases with maternal age and it seems worth noting that the deterioration in fidget viability apparently starts before the major decrease in litter size.

Extensive human data on birth weight and gestation time in relation to maternal age, parity and infant survival have been analysed by Karn and Penrose (1951). They showed that birth weight increases with parity and that weights somewhat less than the maximum were most favourable to survival. Thus probability of survival showed parabolic variation with parity. It is of interest that pallid viability and perhaps also fidget viability show a similar parabolic variation with maternal age. No attempt has been made with these data to separate the effects of maternal age and parity, but the two are, of course, highly correlated. Certainly fidget and, to a lesser extent, pallid reduce birth weight so that an initial increase in birth weight with maternal age may well account for the initial increase in viability. The subsequent decrease is, presumably, due to a general deterioration of the *in utero* environment with increasing maternal age.

4. ANALYSIS OF THE DATA FROM MALE COUPLING HETEROZYGOTES

The smaller body of data that was obtained from male coupling heterozygotes showed no heterogeneity with respect to the proportions of either fidgets or pallids. The data are summarised in table 6.

TABLE 6

Summary of data from male coupling heterozygotes

+	*pa*	*fi*	*pa, fi*	Total
66	19	10	22	117

Recombination fraction : 26·57 per cent.±4·30 per cent.
Fidget viability : 41·88 per cent.±9·67 per cent.
Pallid viability : 79·58 per cent.±18·38 per cent.

All estimates were obtained using a product formula (Fisher, 1935 and later).

The estimate of the recombination fraction, using a product formula and ignoring the possibility of viability interactions, is 26·57 per cent.±4·30 per cent. This is slightly lower than the value given by the female heterozygotes, but the difference is far from significant. Sex differences in this region of linkage group V have been summarised and discussed by Wallace (1957). She pointed out that recombination fractions on the *pa* side of the *fi* locus were lower in males than in females but that her data for the region *fi-Sd*, on the other side of *fi*, gave a

higher recombination fraction in the males. However, the sex difference for the *fi-Sd* region was not significant. Similar differences were obtained by Parsons (1958*a*) using the markers Ragged, agouti, and wellhaarig also on linkage group V. The only significant sex differences (Fisher and Landauer, 1953; Owen, 1953) on this chromosome give a lower value for the males and the data presented here do not contradict this. A significant sex difference with a higher recombination fraction in the males has, however, been reported by Mallyon (1951) for markers on another chromosome. It is in fact well known that variation in recombination, from whatever cause, is generally more marked for regions near the centromere (see, *e.g.* Parsons, 1958*b*). In fact Parsons (1958*b*) used evidence from sex differences in recombination to help in attempting to place the centromere in linkage group XIII of the mouse. It might therefore be expected that a region showing a marked effect of maternal age would be one near the centromere and so would also show a sex difference. It seems worth noting that the data presented here contradict this expectation.

Estimates of the fidget and pallid viabilities using a product formula are 41·88 per cent.$\pm$9·67 per cent. and 79·58 per cent.$\pm$18·38 per cent. respectively. These values show a striking difference from the estimates obtained from the female heterozygote data. The fidget viability is much lower but the pallid viability is higher. The difference in the pallid viabilities does not approach significance but the difference in the fidget viabilities is suggestive giving a significance level of 13·4 per cent. It should be pointed out that all the data which have been presented in this paper refer only to mice which have been classified with certainty for both pallid and fidget. Pallid is easily classifiable at birth. Fidget, however, is not usually classifiable with certainty until the mice are two or more weeks old. The procedure that has been followed, in general, is to do a provisional classification at an early age and to check this later. There is some mortality between these two classifications but it would seem that most of the elimination occurs either *in utero* or at a very early age, perhaps at birth. Mice lost at birth are generally eaten by the parents so that it is not practicable to obtain an estimate of the proportion lost at this stage. The average number of mice per litter which reached the age at which they could be classified with certainty was 4·94 from the female heterozygotes but only 3·34 from male heterozygotes, when the mothers were pallid fidgets. The poorer fertility of the pallid fidget females seems to be associated with a reduced fidget viability and, perhaps, compensated by an increased pallid viability.

5. DISCUSSION

The order of the loci on linkage group V is *a-un-we-pa-fi-Sd*. Data from experiments with the loci *a*, *un*, *we*, *pa* (Fisher, 1949; Fisher and Landauer, 1953; Owen, 1953) suggest a combined map length for the

region *a-pa* of about 16 cm. Wallace (1957) obtained an estimate of about 44 cm. for the *a-fi* map length assuming that interference follows Kosambi's rule (Kosambi, 1944; Owen, 1950). The *pa-fi* recombination fraction of about 27 per cent., reported in this paper, fits in well with these estimates of map length. There is little doubt that the theory of interference first proposed by Fisher, Lyon and Owen (1947) and later elaborated by Owen (1950, 1953 and other papers) provides a useful tool for the construction of genetic maps in an organism like the mouse where it is not possible to cover a chromosome with a series of closely linked markers. However, it seems unlikely that extensive linkage experiments in mice will throw much further light on the phenomena of recombination and interference.

The relevance of the findings on variation in viability and recombination with maternal age to studies on the incidence of human congenital abnormalities has been discussed in a preliminary communication of these data (Bodmer, 1961). Mongolism in man is now known to be caused by aneuploidy for one of the smaller somatic chromosomes (Lejeune, Turpin and Gautier, 1959). This is most probably the result of meiotic non-disjunction for the relevant chromosome. Mongolism is also well known to increase markedly in incidence with increasing maternal age (see, *e.g.* Penrose, 1934). Early work with *Drosophila* showed a clear relationship between non-disjunction and recombination (see Mather, 1938). An increase of non-disjunction is generally associated with a decrease in crossing over and the two have a common origin in the restriction of pairing. Thus the increase in the incidence of mongolism with maternal age in humans, reflecting an increase in the frequency of non-disjunction, and the decrease in the frequency of recombination with maternal age in mice may well have a common origin in the effect of maternal age on the efficiency of pairing at meiosis. In plants Rees and Naylor (1960) have shown that developmental variation within an anther may be associated with highly significant differences in the frequency of chiasma formation. Chiasma formation and chromosome pairing may clearly be considerably affected by developmental differences such as might occur in the ovum of the ageing mother. In fact Polani *et al.* (1960) have suggested a specific mechanism for the way this might happen, in terms of the possible effects of maternal age on the nucleolar organiser which is on the chromosome associated with mongolism.

Many congenital abnormalities of the nervous system in humans are known to increase in incidence with increasing maternal age. (See, *e.g.* Penrose, 1939; Edwards, 1958.) Such developmental abnormalities are generally considerably subject to environmental influences (Edwards, 1958) and cannot be explained by any simple genetic hypothesis. In general it seems unlikely that any congenital abnormality associated with a single gene difference should show an increased incidence with increasing maternal age, for such factors are likely to be associated with fairly severe viability disturbances, although

perhaps not so severe as in mice because of the lack of intra-uterine competition. By analogy with the situation for fidget and pallid in mice, we might expect such viability disturbances to reduce incidence, at least for the later maternal age groups.

The value of data from laboratory animals for the interpretation of the incidence of congenital abnormalities in humans was early pointed out by Wright (1926). This has been emphasised more recently by Holt (1948) and Searle (1959) but still does not seem to be fully appreciated.

It is worth noting that with linked factors the only satisfactory experimental design for obtaining information on viability effects is the balance backcross linkage test. A serious limitation of this sort of data, however, lies in the complicated circumstances which result in an observed viability deficiency and our almost complete ignorance of the physiological and biochemical bases of such genetic defects.

6. SUMMARY

1. Data are presented from a linkage test with the factors pallid and fidget on linkage group V of the house mouse. The data from female heterozygotes are balanced but there are only data from male coupling heterozygotes.

2. There is significant heterogeneity between matings in the ratio of fidgets to non-fidgets but no heterogeneity in the ratio of pallids to non-pallids. This supports the suggestion that there is some measure of genetic control of the fidget viability.

3. The data from female heterozygotes are analysed by the methods of Bodmer and Parsons (1959*a*) and Bodmer (1959) and support a " multiplicative " model.

4. The estimate of the recombination fraction in females (27·44 per cent.$\pm$1·59 per cent.) does not differ significantly from that in males (26·57 per cent.$\pm$4·38 per cent.). The values agree with previous estimates of map length for this region of linkage group V.

5. There is evidence for a significant decrease in recombination fraction with the age of the maternal heterozygous parent. There is also a very significant linear decrease in fidget viability with maternal age. The pallid viability and, to a lesser extent, the fidget viability, show significant parabolic variation with maternal age.

6. Multiplicative estimates of the fidget and pallid viabilities from the female data are 58·68 per cent.$\pm$5·01 per cent. and 64·39 per cent.$\pm$5·50 per cent. respectively. The estimates from the male data are 41·88 per cent.$\pm$9·67 per cent. and 79·58 per cent.$\pm$18·38 per cent. respectively.

7. The relevance of the data on variation in viability and recombination with maternal age to studies on the incidence of congenital abnormalities in humans, is discussed. The value of studies with mice in helping to interpret such phenomena in humans is emphasised.

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FERTILITY STUDIES IN THE FIELD BEAN (*VICIA FABA* L.)

II. INBREEDING

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1. INTRODUCTION

Previous work has indicated that *Vicia faba* is a naturally cross-pollinating species but under field conditions insect pollination appears to be inadequate and much of the seed that is set (up to 70 per cent. of the total) results from self-pollination (Rowlands, 1958, 1960). Moreover, the average proportion of flowers that set in the field is 20-25 per cent., which suggests that even self-pollination is not very successful, in fact data from controlled self-pollination indicate considerable self-sterility.

A hypothesis was put forward in the first paper to explain this self-sterility in terms of homozygosity of deleterious recessive genes which can accumulate in any outbreeding system.

Breakdown of the original outbreeding system resulting from migration of the species from its centre of origin and cultivation practices, coupled with unsatisfactory insect pollination, could be followed by zygotic sterility caused by deleterious recessive genes. The present system is, therefore, one of balance where the pressure to inbreed is counteracted by the requirement for " tripping " of the flowers and zygotic infertility.

From the point of view of yield improvement it would appear that reliance on insect pollination for seed production introduces too much uncertainty, but in common with other cross-fertilising plants, inbreeding in beans results in loss of vigour. Nevertheless for ease of handling, a self-tripping, self-fertile, line would offer great advantages. Inbreeding in field beans is therefore here discussed in relation to the use of pure lines in improvement work.

2. PRELIMINARY STUDIES

The use of a white-flowered line (Ch 170) of field beans enabled a comparison of hybrids and inbreds to be made. Alternate rows of the white-flowered (recessive) and coloured-flowered (dominant) lines were grown and the progeny from the white-flowered line separated into outcrossed or inbred types in the subsequent year. (The white-flowered line originated from a single plant and was already inbred.) The distribution of flower number and pod number per plant in the two groups of material under field conditions is shown in fig. 1.

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In the case of both flowers and pods, a wide variation characterises the outcrosses while this variation is much reduced in the inbreds. Moreover, mean flower and pod number is lower in the inbred than in

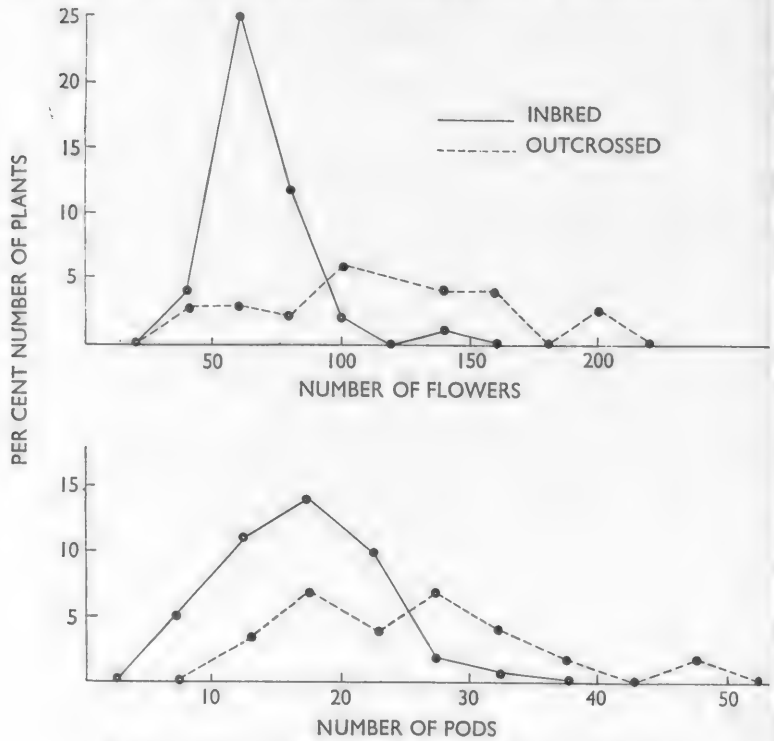

FIG. 1.—Frequency distribution of flowers and pods per plant in inbred and outcrossed bean plants.

the crossbred material. Some of the inbred material did, however, produce more than 20 pods per plant and this suggested further investigation into the behaviour of pod setting during inbreeding.

3. GENERAL INBREEDING STUDIES IN THE GLASSHOUSE

Material was selected for study from six field populations namely:

Ch 170	the white-flowered already inbred line
Ch 4	a commercial stock of tick beans
B 26	a selection from Ch 4
Ch 103/2	a selection from a commercial line (*ex* Dorset)
Ch 35/2	a selection from a commercial line (*ex* Devon)
Cp 145	a high-yielding flower colour selection.

Two samples of five plants were taken from each of the populations, one comprising five very good podding plants, and the other, five plants taken at random. In the case of Ch 170 five additional good plants were taken (A selections) and from the Ch 4 population five of the worst podding plants that could be found. The pod numbers of each of these parent plants are shown in table 1.

TABLE 1

Pod numbers of parent plants for inbreeding experiment (open-pollination, 1955)

Population		Plant reference no.					Mean
		1	2	3	4	5	
Ch 170	Selected	78	70	79	64	117	81·6
	A Selection	55	50	48	51	61	53·0
	Random *	...	...	...	...	...	...
Ch 4	Selected	40	39	35	35	32	36·2
	Random	31	24	24	15	21	23·0
	Worst	11	13	11	11	14	12·0
B 26	Selected	29	44	36	33	70	42·4
	Random	13	24	12	9	30	17·6
Ch 103/2	Selected	37	29	43	56	40	41·0
	Random	33	9	14	23	8	17·4
Ch 35/2	Selected	27	31	38	28	43	33·4
	Random	26	23	10	15	11	17·0
Cp 145	Selected	29	42	28	45	42	37·2
	Random *	...	...	...	...	...	...

* Pod numbers were not recorded for random plants of Ch 170 and Cp 145.

Five progeny plants from each original parent were grown in an insect-proof glasshouse during 1956 and pod and seed number per plant recorded on each individual. As many flowers as possible were artificially manipulated to simulate the effect of tripping, so that the data obtained reflected the capacity of each plant to set selfed seed.

The most significant point in table 2 is the low pod-setting on selfing as compared with the performance of the parental lines under open-pollination. This progeny performance on selfing is not correlated with the parental pod set, and their self-fertilities are all of the same order. In general, the selected material would appear to possess lower self-fertilities than the random material. This is to be expected if the selected parents are highly podding by virtue of heterozygosity for self-sterility genes, and this superiority would not be apparent in their progeny. Despite the rather low mean pod number per plant, most of the lines contained occasional plants producing 15-20 pods, which may be regarded as an adequate set in the field. Seed production

per plant, and also the mean number of seeds per pod, are reduced, selected plants again suffering more than random plants.

TABLE 2

Mean pod and seed setting of parental lines and S_0 segregates from each (1956)

		Parental pod mean (open-pollination)	Pods per plant on selfing S_0 segregates			Seeds per	
			Mean	Range	Per cent. of parent	Plant	Pod
Ch 170	Selected	81·6	7·2	0-20	8·8	14·2	1·9
	A Selection	53·0	9·7	4-22	18·3	...	...
	Random	...	5·6	0-28	...	12·3	2·2
Ch 103/2	Selected	41·0	9·8	0-29	23·9	20·4	2·1
	Random	17·4	10·2	0-26	58·6	21·6	2·1
Ch 35/2	Selected	33·4	7·0	2-28	21·0	14·7	2·1
	Random	17·0	10·0	4-23	58·8	20·6	2·1
B 26	Selected	42·4	6·7	0-13	15·8	11·8	1·8
	Random	17·6	7·7	1-15	43·8	14·9	1·9
Ch 4	Selected	36·2	7·8	1-19	21·5	12·1	1·6
	Random	23·0	8·1	0-20	35·2	15·1	1·9
	Worst	12·0	6·2	1-12	51·7	14·3	2·3
Cp 145	Selected	37·2	6·0	0-10	16·1	15·0	2·5
	Random	...	6·6	1-21	...	15·3	2·3
Mean		34·3	7·8	...	31·1	15·6	2·1

From this population, those plants bearing 20 or more pods or more seeds per plant were selected. Fifty seeds of each of the eight highest lines were sown, together with five seeds of each of seventeen fairly good lines. In addition, five seeds of each of the 22 progenies of Ch 4 were sown. These plants were allowed to set seed without the aid of " tripping " and an estimate of spontaneous self-fertility was obtained. These results are presented in table 3.

Pod number per plant is lower than the corresponding progeny mean in 1956, and very much lower than in the selected parental S_0 plants. Seed number per plant is reduced and so is the percentage of flowers setting pods, and the mean seed number per 100 flowers. Some of the progenies contain a high proportion of sterile plants; Ch 103/2 progenies in general were worse than the others, and Ch 103/2R3/2 were worst of all, with over 50 per cent. of the plants sterile. Least affected by inbreeding is Ch 170 R 5/2 with fewest sterile plants and highest pod and seed sets. The family Ch 170 is based on a single white-flowered plant and is already somewhat inbred. Ch 170 R 5/2, Ch 35/2 R 3/1 and Ch 103/2 S 2/4 are perhaps the most profitable progenies to follow up in attempting to select for self-fertility.

TABLE 3

Mean self-fertility of S_1 progenies from highly self-fertile S_0 plants (1957)

Progeny	No. of plants grown	No. of pods on S_0 plants	Per cent. sterile plants	Mean no. per plant of			Mean no. of seeds per pod	Per cent. flowers forming pods	Seeds per 100 flowers
				Flowers	Pods	Seeds			
Ch 170 R 5/2 .	32	28	3	125	5·7	9·6	1·7	4·6	7·7
Ch 35/2R3/1 .	44	23	5	119	4·8	7·5	1·6	4·0	6·3
Ch 35/2R4/1 .	43	22	14	127	4·3	6·0	1·4	3·4	4·7
Ch 35/2S3/2 .	49	28	16	125	3·9	5·1	1·3	3·1	4·2
Ch 103/2R1/1 .	37	21	27	140	2·4	2·4	1·0	1·6	1·6
Ch 103/2R4/1 .	41	26	32	133	2·1	2·5	1·2	1·6	1·9
Ch 103/2R3/2 .	38	24	58	98	3·9	1·0	0·3	4·0	1·0
Ch 103/2S2/4 .	42	29	17	109	5·4	9·1	1·7	5·0	8·4
Mean . .	...	25·1	21·5	122	4·1	5·4	1·3	3·4	4·4

TABLE 4

Pod and seed setting in S_1 progeny plants and S_2 families of Ch 170 R 5/2 (1958)

Progeny no.	S_1 progeny plants			S_2 family means		
	No. of			No. of		
	Pods	Seeds	Seeds per pod	Pods	Seeds	Seeds per pod
1	3	4	1·3	12·5	21·5	1·7
2	18	35	1·9	2·4	4·2	1·8
3	1	2	2·0	2·0	3·5	1·8
4	6	9	1·5	8·0	17·0	2·1
5	2	2	1·0	4·5	8·0	1·8
6	6	11	1·8	5·0	7·8	1·6
7	2	4	2·0	6·0	11·7	2·0
8	14	17	1·2	5·0	8·0	1·6
9	10	17	1·7	9·3	19·7	2·1
11	1	1	1·0	2·7	4·3	1·6
13	9	18	2·0	9·5	16·0	1·7
14	5	5	1·0	8·0	12·7	1·6
16	6	8	1·3	0·5	0·5	1·0
17	4	8	2·0	2·3	4·0	1·7
18	7	15	2·1	3·3	6·0	1·8
20	4	7	1·8	6·5	16·0	2·5
21	16	27	1·7	10·0	17·0	1·7
22	9	15	1·7	3·3	6·8	2·1
23	5	11	2·2	3·0	6·3	2·1
24	3	8	2·7	17·0	34·0	2·0
25	7	10	1·4	15·0	32·0	2·1
26	3	1	0·3	9·0	10·0	1·1
27	3	3	1·0	2·0	3·5	1·8
28	4	10	2·5	5·4	10·6	2·0
29	1	2	2·0	18·0	38·0	2·1
30	5	6	1·2	11·5	18·0	1·6
31	11	19	1·7	7·3	11·0	1·5
32	2	5	2·5	6·3	14·3	2·3
Mean .	5·9	10·0	1·7	5·9	11·0	1·8

In contrast to the above material the less highly fertile S_0 progenies were only scored for pod and seed number but the data were comparable with those obtained from the more highly fertile S_0 plants.

Progenies from Ch 103/2 were again generally of lower self-fertility, while Cp 145 S 2/3 was highest in this group, but taken as a whole the group showed no lower pod and seed set than the progenies from highly fertile S_0 plants.

Line Ch 170 R 5/2 was continued in its entirety in 1958, five segregates representing each of the S_1 progeny from the previous year. Of

TABLE 5

Pod and seed setting in S_3 progenies of Ch 170 R 5/2

Progeny no.	S_3 progeny			No. of plants per S_3 progeny
	Mean no. of			
	Pods	Seeds	Seeds per pod	
1/2	3·1	5·4	1·7	15
4/1	4·1	7·5	1·8	14
7/2	2·2	3·4	1·5	12
9/1	6·4	11·6	1·8	15
9/2	9·7	18·6	1·9	12
13/1	1·4	2·4	1·7	3
20/1	4·7	10·5	2·2	15
21/22	11·4	23·5	2·1	11
21/1D	11·6	28·2	2·4	10
10/1D	6·0	12·4	2·1	8
24/1	2·5	4·8	1·9	15
25/1	5·4	9·9	1·8	13
29/1	5·6	10·0	1·8	15
	5·7	11·4	1·9	...

the other lines, five plant progenies were sown of Ch 35/2 R 3/1 and Ch 103 S 2/4 and, in addition, five plant progenies (where seed permitted) of the highly self-sterile line Ch 103/2 R 3/2. Pod and seed number per plant were again recorded and the data for Ch 170 R 5/2 are given in table 4.

Pod and seed number per plant are slightly improved in 1958 although no conscious selection has been practised for this factor. If, however, the 1958 population had been based only on those plants with 16 or more seeds, the means for pods and seeds would be 7·3 and 12·6 (compared with 5·9 and 11·0 for unselected plants). In this family, selection would have improved self-fertility. In Ch 35/2 R 3/1 and Ch 103/2 S 2/4 where some selection was in fact practised the means were maintained in the former (4·1 for pods and 7·3 for seeds) while they fell in Ch 103/2 S 2/4 (to 3·0 for pods and 6·0 for seeds). The low fertility line Ch 103/2 R 3/2 had a lower pod set (0·97 per

plant) but maintained seed setting at 1·6 per plant, with again over 50 per cent. self-sterile plants.

The 1959 material was based on progenies from S_2 plants which bore over 20 seeds. S_3 progeny means for pods and seeds per plant and seeds per pod are given in table 5.

TABLE 6

Pod and seed setting in parent plants, S_0 plants and S_1 progenies of Ch 4

Line and progeny no.	Pod no. of		Means of S_1 progenies (1957)						
	Parent plants (1955) (open-pollination)	S_0 plants (1956) (tripping)	Flowers	Pods	Seeds	Per cent. flowers set	Seeds per 100 flowers	Per cent. flowers set	Seeds per 100 flowers
Ch4R1/1		13	108·2	5·4	9·0	5·0	8·2		
2/1		6	102·0	2·0	3·0	2·0	3·2		
3/1	31	11	100·2	3·0	5·2	3·0	5·3	4·4	8·5
4/1		6	101·0	14·0	36·5	13·9	36·1		
5/1		9	107·0	4·2	7·0	3·9	6·3		
1/2		2	109·0	4·0	6·0	3·7	4·8		
2/2		5	72·0	1·8	3·0	2·5	3·8		
3/2	24	10	100·8	0·6	1·6	0·6	1·5	1·6	2·5
4/2		2	101·4	1·2	1·2	1·2	1·3		
5/2		10	85·4	0·8	1·6	1·0	1·9		
1/3	24	8	106·0	0·6	1·0	0·6	0·9	1·9	3·9
4/3		10	106·8	3·4	7·2	3·2	6·9		
1/4		17	98·4	6·6	9·0	6·7	9·1		
2/4		20	104·6	5·2	7·0	4·0	5·8		
3/4	15	16	92·6	4·0	6·2	4·3	6·8	6·7	13·5
4/4		13	109·0	10·4	15·2	9·5	14·4		
5/4		6	106·3	9·3	17·0	8·7	15·9		
1/5		5	101·6	1·4	2·0	1·4	2·0		
2/5		6	136·6	4·8	4·0	3·5	3·1		
3/5	21	7	103·4	3·6	6·2	3·5	6·1	2·8	4·4
4/5		12	110·6	1·0	2·0	0·9	1·8		
5/5		8	100·8	4·6	9·0	4·6	9·0		
Mean .	23·0	9·2	102·9	3·8	6·4	4·0	7·0	...	...

It will be observed that the mean number of pods, seeds, and seeds per pod, for the whole Ch 170 population has not been improved by the particular form of selection practised. However, two families stand out as consistently high pod and seed setters, namely, 9 and 21 (tables 4, 5), and of these No. 21 is extremely promising, but neither family could have been recognised in S_1 or S_2 as likely to give consistently self-fertile progeny.

It would appear that no general correlation exists between individual plants and their progenies with respect to pod and seed number.

4. INBREEDING OF CH 4

The original field population was analysed as fully as possible in 1955. Forty plants within the population were marked at random and the numbers of flowers and pods were recorded throughout the season.

TABLE 7

Analysis of variance of differences between lines and also between progenies with respect to seeds per 100 flowers

Component	N	Mean squares	F	Probability
Between lines . .	4	242·0	20·1	<0·1 per cent.
Between progenies .	22	153·8	12·7	<0·1 per cent.
Within progenies .	76	12·1	...	...
	102			

TABLE 8

Pod and seed setting in S_1 progeny plants, S_2 families and S_3 families of Ch 4

Progeny and family no.	S_1 plants (1957)		S_2 families (1958)			S_3 families (1959)		
	No. of		Mean no. of			Mean no. of		
	Pods	Seeds	Flowers	Pods	Seeds	Flowers	Pods	Seeds
4 1 (1)	13	35	59·4	3·7	9·5	71·1	4·5	9·5
(2)	15	38	67·2	5·3	11·9	55·0	8·0	18·5
1 2 (2)	2	3	203·7	1·7	2·7	83·0	0·8	1·3
(3)	10	15	135·6	4·3	7·4	63·8	3·6	7·0
4 4 (5)	14	18	111·9	4·8	9·1	65·2	5·9	10·1
5 4 (4)	19	30	90·1	2·9	4·6	67·7	3·2	4·9
2 5 (3)	8	11	75·2	4·8	8·8	54·5	4·9	9·1
Mean .	11·6	21·4	106·2	3·9	7·7	65·8	7·7	8·6

The number of flowers per plant varied from 50-150 with a mean of 98·0. The pods had a much more restricted distribution, namely, from 5-50 per plant with a mean of 23·1. Between 10 and 50 per cent. of the total flowers set pods, the mean flowers set being 23·6 per cent.

Progenies were grown from each of the five random plants described in table 1 and allowed to set seed after tripping in an insect-proof glasshouse. The data on selfing this Ch 4 population are given in table 6.

Pod number although reduced in most S_0 plants was still high in some, particularly in those derived from Ch 4 R 4. In 1957, random progenies were grown from each original line and they were allowed to set seed unaided in an insect-proof glasshouse. Pod number was still further reduced, except in Ch 4 R 4 progenies and in line 4/1 where a considerable increase occurred. An analysis of the differences between progenies and lines on the basis of seeds set per 100 flowers is given in table 7.

Lines Ch 4 R 1 and Ch 4 R 4 are significantly better than the others and differences between progenies within lines are also significant, progenies 4/1, 4/4 and 5/4 being particularly self-fertile.

Progenies 4/1, 1/2, 2/4, 4/4, 5/4 and 2/5 were continued in 1958 and 1959, and all the available seed was sown up to a maximum of 20 per progeny. These plants were again allowed to set seed unaided in an insect-proof glasshouse; the number of flowers, pods and seeds per plant were recorded (table 8).

The mean numbers of pods and seeds again increased in 1959, also the percentage of flowers forming pods. This increased flower setting cannot be due to the elimination of self-sterile plants, as can be seen from the following figures:

Year	Generation	Per cent. flowers setting pods	Per cent. sterile plants
1955	Open-pollinated parents	23·6	0·0
1956	S_0 selfed plants (tripped)	7·3	5·6
1957	S_1 " "	3·7	19·4
1958	S_2 " "	5·1	2·1
1959	S_3 " "	11·7	10·4

Although the reduced number of flowers per plant in 1959 may be partly responsible for the relative increase in flower setting, nevertheless this improvement is a real effect and not a chance occurrence. This is shown by the analysis of the correlations of S_3 on S_2 family means for number of pods and seeds per plant. For pods $r = +0{\cdot}91$ and for seeds $r = 0{\cdot}94$, both correlation coefficients are highly significant (P = 0·1 per cent). Not only do the families and progenies differ significantly from each other in self-fertility but these differences appear to be inherited, and therefore are capable of improvement by selection. Some improvement in seed number per plant is shown but no conscious selection has been made for seed-setting on selfing. In fact it would appear that as soon as direct selection is made for seed-setting (as in Ch 170) the actual seed-set may be reduced.

5. DISCUSSION

The behaviour of the two populations Ch 4 and Ch 170 under inbreeding offers an interesting comparison. The families were grown alongside each other every year and the only difference between them

was that Ch 4 was based on unselected progenies in each generation while the Ch 170 population was subjected to intensive selection for high seed-set:

	S_0	Mean seed-set in each generation		
		S_1	S_2	S_3
Ch 170 . . .	13·3	5·4 (6·8)	11·0	11·4
Ch 4 . . .	13·8	6·4	7·7	8·6

The flowers in the S_0 generation were hand "tripped" and this accounts for the larger seed-set. In the S_1 generation the highly-selected Ch 170 progenies had a lower seed-set (5·4) than those where selection had been relaxed (6·8 seeds). Despite the intensive selection seed-set was only maintained in the S_3 generation of Ch 170 while the unselected Ch 4 progenies continued to improve in seed-set.

Genetic variability for seed-setting on selfing is undoubtedly present in the material since the lines here diverged on inbreeding (table 7) although the grand mean has remained little changed throughout. Sybenga (1958) has described an experiment in rye where a record was made of seed-setting on self-pollination during inbreeding. Seed-set improved in S_2 and S_3 generation but decreased in the S_4 and S_5 generations. Moreover, seed-set on selfing never exceeded that set after free pollination and only approached it in the S_3. Similar situations were observed on inbreeding grasses and other forage crops (Wexelsen, 1952) and particularly in red clover (Thomas, 1955).

Thompson and Rees (1956) demonstrated that during inbreeding of rye some selection occurred for heterozygotes. Selection did not operate in favour of heterozygotes in general, but in favour of a particular heterozygous combination, indicating that heterozygosity in itself is not necessarily advantageous. It was not possible to rule out completely a recessive gene of major effect, but it was suggested that a more likely explanation was that of a superior balance of polygenes in that particular hybrid combination.

In part I of the present investigation (Rowlands, 1960) infertility at the zygotic level was explained in terms of homozygosis of deleterious recessive genes following inbreeding, and, moreover, that these genes were linked together in a balanced system. It is inevitable with only six linkage groups that other balanced combinations will be associated with these linked deleterious recessives and will contribute to the superiority of certain heterozygous combinations. The selective advantage in natural populations of the linkage within and between polygenic systems has been discussed by Mather (1953). Correlated responses to selection can be extensive under such conditions and can

give rise to true inertia (or homeostasis) but although they slow down, they do not finally prevent response to selection.

Evidence for dominance and non-allelic interaction of the genes controlling self-fertility was obtained from the diallel analysis of seed-setting in part I, and Jinks (1955) has related similar genetic situations in maize and other crops to close linkage in opposition of particular gene combinations. In such a system, inbreeding will fix the balanced combinations (Mather, 1953) and since the phenotypes determined by the different balanced combinations are alike they will be retained equally in the population. Effective segregation and recombination cannot occur in the absence of crossing, so that the balanced combinations will not be broken up despite the presence of genic differences. When the variability has been frozen in this way, change under directional selection is impossible and recombination must be induced by crossing over for any progress to take place in the population.

Breese (1956) has discussed assortative (*i.e.* like by like) mating in this context and concludes that " where genes controlling a character tend to be linked in opposition, assortative mating followed by selection, by avoiding the premature homozygosis and fixation resulting from inbreeding should maximise the chances of securing and ultimately fixing desirable recombinants ". Such a programme of assortative mating and selection in beans might well result in the isolation of vigorous self-fertile lines in which a new internal chromosomal balance may be established.

The data presented by Williams and Gilbert (1960) show that such internal balance has been achieved unconsciously in the tomato during its change-over from outbreeding to inbreeding. Consequently, once the underlying genetic system has been discovered it is reasonable to suppose that a properly designed breeding programme should be able to complete quickly and effectively the change-over to inbreeding that may have begun naturally in any outbreeding species.

6. SUMMARY

1. The behaviour of pod and seed-set under self-pollination has been investigated during a programme of inbreeding.

2. Self-pollination was relatively ineffective in producing seeds, and selection for this character over three generations of inbreeding was not successful. Intensive selection for seed-set on self-pollination in one population had no marked effect, while in an unselected population seed-set appeared to improve slightly after three generations.

3. It is suggested that the presence of deleterious recessive genes, coupled with the development of close linkage in opposition of particular gene combinations, has produced a balanced system which directional selection alone is unable to break up.

4. Assortative mating followed by selection, by avoiding the fixation resulting from inbreeding, should maximise the chances of securing

and fixing desirable recombinants, and such a programme is recommended for the development of vigorous self-fertile lines not only in field beans but also in other outbreeding crops.

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NOTES AND COMMENTS

MOSAICISM IN MAN, INVOLVING THE AUTOSOME ASSOCIATED WITH MONGOLISM

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Examples of chromosome mosaicism, that is, the presence in one individual of cells with different chromosome constitutions, have been described recently in man. Ford *et al.* (1959*b*) and Hayward (1960) both found XXY and XX cells in males diagnosed as Klinefelter syndrome. Ford (1960) reported the finding of XO and XX cells in females diagnosed as Turner's syndrome, and Jacobs *et al.* (1960) found XXX and XO cells in a female with primary amenorrhœa. Blank *et al.* (1960) and Hirschhorn *et al.* (1960) both found XY and XO cells in subjects with evidence of hermaphroditism. All of these examples involved the sex chromosomes only, and otherwise showed a normal complement of 22 pairs of autosomes. An autosomal mosaic has recently been described by Clarke *et al.* (1961) who found normal cells and cells which were trisomic for chromosome 21 in a mongoloid child.

Böök *et al.* (1959), Ford *et al.* (1959*a*), Lejeune *et al.* (1959) and Jacobs *et al.* (1959) found that mongolian idiots all have a small acrocentric chromosome additional to the normal complement. This chromosome is generally agreed to be number 21 in the Denver classification (*Lancet*, 1960 (i), 1063). The trisomy of this chromosome, causing mongolism, probably has its origin, in most cases, from primary non-disjunction during oögenesis (Jacobs *et al.*, 1959) forming an egg cell with two chromosomes 21, which is fertilised by a normal sperm.

The present report is of a case of mosaicism involving autosome 21, which is unique in showing three different complements of this chromosome.

1. CASE REPORT

The subject, a 51 year old male, has always been regarded as a mongol, although he shows some atypical mongoloid traits. His stature is short. The back of his head is flat; the eyes, which are widely separated by a broad nasal bridge, do not have epicanthal folds or the typical mongoloid slant; the ears are large, but of normal shape and the tongue is of normal size. His chest is flat rather than rounded; there is no clinical evidence of congenital heart defects. The hands and feet are short and broad; the left hand shows a " simian crease ", but the creases of the right hand and the feet are normal. The subject shows a very severe grade of mental deficiency and is unable to speak or to obey simple instructions, although he can feed himself. His mother was 38 years of age at the time of his birth.

2. METHODS

Leucocytes from peripheral blood were cultured, treated with colchicine and hypotonic saline by a modified form of the method of Moorhead *et al.* (1960). The cells were spread by air drying, stained with a May-Grunwald, Giemsa combination and mounted in *Depex*. This technique gives many well spread mitotic figures with a minimum of damage or chromosome loss, and allows accurate counting and identification of the chromosomes.

3. CHROMOSOME STUDIES

The chromosomes were counted in 101 well spread and undamaged cells with the following results :

Chromosome number . .	41 - 46	47	48	Total
Number of cells . . .	1	42	53	5	101

These results contrasted sharply with those from other chromosome studies we have made by the same method, all of which showed a single modal number with little variation from it. Twenty-nine cells with 46 chromosomes, 39 cells with 47 chromosomes and 3 cells with 47 chromosomes were analysed in detail with special regard for the number of small acrocentric chromosomes present. A normal male shows 5 of these chromosomes : 2 of number 21, 2 of number 22 and the Y chromosome. The cells with 46 chromosomes all contained 5 small acrocentric chromosomes, those with 47 chromosomes all contained 6 small acrocentric chromosomes, and the 3 cells with 48 chromosomes all showed 7 small acrocentric chromosomes.

These results clearly indicate mosaicism for one of the small acrocentric chromosomes, and the mongoloid facies of the subject is strong evidence that this is chromosome 21.

4. DISCUSSION

Mosaicism in an individual will result from secondary non-disjunction of a chromosome in a post-zygotic mitosis. The present triple stem-line mosaic is not likely the result of non-disjunction of chromosome 21 during the divisions of a normally constituted zygote with 46 chromosomes, because if this occurred during the first cleavage division, 45 and 47 chromosome stem-lines would result, and if during the second or later cleavage divisions, 45, 46 and 47 chromosome stem-lines would result. Assuming a zygote which is trisomic for chromosome 21, that is a " mongoloid zygote ", non-disjunction of chromosome 21 in the first cleavage division would give 46 and 48 chromosome stem-lines, whereas non-disjunction in the second or a later cleavage division would give stem-lines with 46, 47 and 48 chromosomes, as were observed. It is most probable therefore that the present mosaic is the result of non-disjunction of chromosome 21 in the second or a later cleavage division of a zygote which was trisomic for this chromosome because of an earlier non-disjunction during gametogenesis. The high frequency of cells with 47 chromosomes supports this view, because non-disjunction of chromosome 21 in one of the cells during the second cleavage division, for instance, would produce a 4-cell stage made up of two cells with 47 chromosomes one cell with 46 chromosomes and one cell with 48

chromosomes. However, such a ratio of different stem-lines might not be maintained in consequent divisions, and Ford *et al.* (1959*b*) consider that stem-lines which are nearer normality may have a selective advantage. Such a view accords with the increase in frequency of the normal 46 chromosome stem-line relative to the abnormal 47 chromosome one, and the decrease in frequency of the more abnormal 48 chromosome stem-line observed in the present mosaic.

Ford *et al.* (1959*b*), Ford (1960) and Hayward (1960), postulate similar origins for the XXY/XX and XO/XX mosaics, that is, mitotic non-disjunction in the second or later cleavage divisions of abnormal XXY and XO zygotes. Hayward considers that the presence of an unbalanced genotype, as in the XXY Klinefelter syndrome, might make mitotic non-disjunction more likely. If these mosaics arose in this way, other stem-lines must have been produced as a result of the secondary non-disjunction. Cells with 44 autosomes and no sex chromosomes in the Turner's mosaics presumably would not have been viable. A 48 chromosome stem-line with the XXYY constitution would have been produced in the Klinefelter mosaic, but neither Ford nor Hayward found evidence of it. Its absence may be surprising, for this chromosome constitution is by no means inviable as is seen from the report (Muldal *et al.*, 1960) of a case of Klinefelter syndrome with the XXYY genotype. However, it is possible that the 48 chromosome stem-line was at some selective disadvantage and was either eliminated or present at too low a frequency to be included in the samples studied. Ford *et al.* (1959*b*) outline evidence that mammalian cells with different visible chromosome constitutions reproduce at different rates, and that cells with a normal chromosome constitution have a selective advantage. Another factor which may explain the failure to find the 48 chromosome stem-line is illustrated by the different chromosome analyses obtained from different tissues of the XXX/XO mosaic by Jacobs *et al.* (1960). Cultures of leucocytes and skin cells showed modal stem-lines of 45 and 47 chromosomes, whereas cultures of sternal marrow contained only cells with 45 chromosomes. They consider this is likely due to the marrow consisting only of cells with 45 chromosomes, whereas the lymph nodes and other sites in the reticulo-endothelial system may contain cells of the other type. Chromosome preparations from bone marrow cells only were examined in both of the Klinefelter mosaics.

Jacobs *et al.* (1960) consider that the most likely explanation of the XXX/XO mosaic is non-disjunction of the X chromosomes during the first cleavage division in a normally constituted XX zygote. The XY/XO mosaics of Blank *et al.* (1960) and Hirschhorn *et al.* (1960) are presumably due to loss, or non-disjunction, of a Y chromosome in an early cleavage division of a normal XY zygote. The 61 per cent. and 41 per cent. frequencies of the 45 chromosome XO stem-line observed in these mosaics are difficult to reconcile with the view of a selective advantage of cells with the normal chromosome constitution.

Stern (1960) has recently reported a mosaic, in *Drosophila*, which like the present mosaic consisted of three types of cells. It is believed that non-disjunction of the X chromosomes, in an early cleavage mitosis of an XX zygote, produced stem-lines having X, XX and XXX constitutions. Non-disjunction occurred during a later cleavage division than in the case of Jacobs *et al.* (1960).

5. SUMMARY

The cells from a leucocyte culture of a man presenting mongoloid features showed distinct stem-lines of 46, 47 and 48 chromosomes, which were considered to be disomic, trisomic and tetrasomic respectively for chromosome 21. This mosaicism probably resulted from mitotic non-disjunction of chromosome 21 in the second or a later cleavage mitosis of a zygote which was already trisomic for this chromosome.

Acknowledgments.—We are indebted to Dr C. S. Moore, Medical Superintendent, Seacliff Hospital, and his staff, for their co-operation; and to Professor E. F. D'Ath, University of Otago Medical School for supporting this study.

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CONDITIONING IN *ARABIDOPSIS THALIANA*

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The importance of the environment in determining the phenotype of an individual has been known since Johannsen's (1909) experiments with *Phaseolus vulgaris*, in which he demonstrated that the phenotype expressed was the result of the interaction between genotype and environment. Recently, however, it has been realised that the effects of the environment may in fact be more persistent. Where this persistence is complete, the progeny from individuals which themselves expressed a characteristic phenotype under a given set of environmental conditions, exhibit this phenotype even after the removal of these specific conditions. Such progeny are then said to have been " conditioned ". The most outstanding example of this has been found in an inbred line of flax by Durrant (1958) and Durrant and Tyson (1960) who used all possible combinations of nitrogen, phosphorous and potassium fertilisers as the conditioning treatments. Results of this nature have obvious agricultural implications and it is therefore desirable to determine which plants can be similarly conditioned.

Arabidopsis thaliana, an automatically self-pollinated ephemeral of widespread distribution in waste places throughout the British Isles, was the species chosen for a series of experiments designed to test whether fertiliser alone or in combination with photoperiodic treatment could induce any permanent effect.

Using fertiliser alone, 20 pots of a highly inbred strain were sown in each of 2 treatments, one being John Innes potting compost with all nutrients added. The other was without nutrients until it was found that 8 of the 20 plants within this group were dying, whereupon 5 mls. of 0·01 concentration of Langridge's solution (1957) was supplied to these plants. All plants in this and subsequent experiments were recorded for flowering-time, leaf number, rosette diameter and final height.

Of the 40 initially treated plants, 16 (8 from each treatment) were selected for further experiments. These 8 plants from within each treatment were then further subdivided according to the degree of expression of the characters recorded into high, medium and low. Prior to sowing, all seed from the selected lines, together with seed from the original race to be used as controls, was subjected to two cycles of temperature treatment to break its dormancy. The experiment, which was laid down in a glasshouse, was of a randomised block design with six replicate-blocks. Nutrient status and day length were the same for all plants. Re-randomisation was carried out at regular intervals in order to distribute edge effects at random.

The 16 selected lines were also put through a second generation of conditioning to fertiliser treatment.

Subsequent experiments in this series, involving seed from plants which had had one generation of conditioning followed by a generation of growth under uniform conditions and from plants which had received two successive generations of conditioning, were designed on a basis similar to that outlined above, with only minor variations in the number of lines and replications used.

For the experiments combining fertiliser and photoperiodic treatments, an ALL or NONE fertiliser treatment was used in all four possible combinations with short or long day-length (8 and 16 hours respectively). Two strains were used in the initial treatments. From each of these four environments three lines per strain were selected for further experiments. This seed and the control seed obtained from the original strains were subjected to the treatment for breaking dormancy. Each of the 24 lines (12 per strain) was used twice in any one replicate, once with and once without fertiliser,

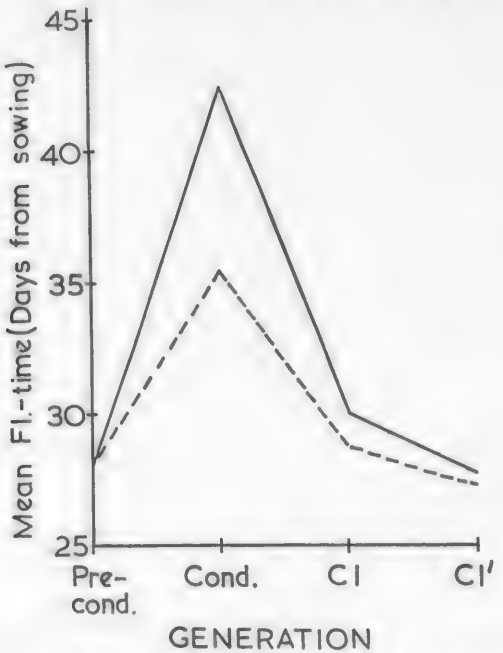

FIG. 1.—Relationship between flowering-time and generation of conditioning for the Langridge strain of *A. thaliana*. The solid line represents the mean flowering-time of the group which did not receive fertiliser, the broken line the mean flowering-time of the group which did receive fertiliser. Generations used were plants prior to treatment, during treatment and 1 and 2 generations removed from treatment respectively.

whilst for the controls, 3 from each strain were grown in each of the 2 fertiliser treatments per replicate. The experiment, which was of a randomised block design with five replicate-blocks, was laid down in a glasshouse. Supplementary light was provided such that all plants received 16 hours of light a day. The whole experiment was surrounded by a guard row.

The results obtained from these experiments indicated that neither of the two sets of treatments applied produced any permanent effect in *A. thaliana*. There was a decrease in the differences between treatment groups which was correlated with the number of generations of growth under uniform conditions (fig. 1). This decrease was found to be more rapid for differences attributable to light than for those produced by fertiliser

alone. Transmission of effect from parent to the early stages of the growth of its offspring, which was thought to be due to the operation of a mechanism affecting the maternal nutrition of the seed, could be seen from data on growth-rates, where differences between groups at any one time could be related to differences in emergence, and from the establishment of a positive correlation between flowering-time of parent and mean germination-time of offspring despite the application to the seed of a treatment to break dormancy.

It is clear from the results that, within the specific experimental conditions described, the system of inheritance operating in Durrant's flax was not demonstrable in *A. thaliana*. There may be various reasons for this failure. In particular, it is possible that, because *A. thaliana* is an ephemeral plant and therefore makes only modest demands on its external environment, the range of treatments employed were not drastic enough to produce any permanent detrimental effect. Although no permanent effect was produced, it was possible to condition the plant and produce differences which persisted for two generations (see fig. 1). This, therefore, seems to indicate that a system similar to Dauermodification was operating. Turning to plants with a longer life-cycle, it is possible that Durrant's success with flax may be due at least in part to the fact that this plant is relatively unimproved. For this reason experiments are now proceeding in an attempt to condition an inbred line of *Nicotiana rustica*, a species about which a considerable amount of information exists regarding the inheritance of variation under major genic or polygenic control.

Acknowledgments.—I wish to express my thanks to Professor K. Mather, F.R.S., and to Dr J. L. Jinks for their helpful advice and encouragement throughout.

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REVIEWS

PRINCIPLES OF PLANT BREEDING. By R. W. Allard. London, New York: John Wiley. 1960. Pp. 485. 72s.

Professor Allard has presented a neatly integrated account of the scientific principles and practice of plant breeding. The main part of his book analyses the genetic architecture of self-fertilising and cross-fertilising populations and appropriately relates the breeding procedures adopted by man to the natural breeding habits of the plants. It is the first comprehensive textbook of plant breeding which gives this important relationship the emphasis it deserves. The apprentice may therefore learn to appraise the applicability, limitations and the reason underlying the various and sometimes devious methods of selection in plant improvement.

The special features of breeding for disease resistance are dealt with separately. Complementary gene systems conferring pathogenicity on the parasite and resistance on the host which permit such remarkable precision in the production of varieties with specific qualities of resistance are well illustrated. In this field at least the breeder can be forearmed against the exigencies of the grower.

Selection within the limits of naturally occurring variation is still the predominant means of plant improvement despite the fact that some of the main patterns of natural variation can now be copied at will by the breeder. Manipulation of numbers and associations of chromosomes and induced changes in the genes themselves have been accomplished. The more delicate adjustments which follow these radical alterations in nature and which restore balance to the changed systems can also be accumulated by artificial selection but much more slowly and with much less precision than the initial large changes. Professor Allard deals with problems of polyploidy, interspecific hybridisation and mutation in turn, reviewing some of the contributions they have made and cautiously summing up their value as general breeding tools. They have not perhaps achieved the over-riding success predicted at an earlier date by their more ambitious proponents; but, applied with understanding and ingenuity, these techniques undoubtedly lend versatility and increased scope to plant breeding.

In *Principles of Plant Breeding* we have a well balanced up to date book which is indeed based on sound principles backed up by suitable examples and well selected references. It is the best so far.

LESLIE K. CROWE.

THE CHROMOSOMES. By M. J. D. White. London: Methuen. 1961. 5th edn. Pp. 188, 26 text figs. 15s.

The fifth edition of this book appears a quarter of a century after the first and at a time, as White puts it, when we are more likely to speak of "the transfer of genetic information" than of "the functioning of the genes". Like the earlier editions, the present one is intended as "an elementary account" which can serve as an introduction to "larger works such as C. P. Swanson's *Cytology and Cytogenetics* and the author's *Animal Cytology and Evolution*".

Since this is the purpose of the book it must be viewed in this light. And since terms like chromatid and nucleolus are defined we can assume that the book is intended for the beginner. How useful will he find it?

The book, as the dust cover tells us, deals with " the structure and behaviour of the chromosomes in cell division and as the chemical basis of the genetic system. Special sections deal with the chemistry of chromosomes, the mechanism of meiosis, the giant salivary gland and lampbrush chromosomes, the chromosome mechanism of sex determination, the role of chromosome rearrangements in speciation and evolution . . .".

This is rather a lot to cover in 170 pages of text each measuring $6\frac{1}{2} \times 4$ inches. But one has little to object to in the subjects chosen. I would, however, criticise the manner and the order in which they are considered.

For example, the reader (for whom the term chromatid has to be defined) is expected to appreciate the autoradiographic studies on chromosome replication (with its implications of " old " and " new " strands and half-chromatids) before he has been given an account of mitosis or even his definition of a chromatid. On reaching the account of mitosis (nine pages later) what do we find?

We find that the elementary student while being given the long-awaited account of the mitotic sequence is expected, at the same time, to accommodate somatic pairing, breakage in and competition between nucleolar organisers, split and multipolar spindles and even " chromatic " elimination. That he is confronted also with the effects of colchicine comes as no surprise because he has already been faced with this in the autoradiographic studies sixteen pages earlier.

The unsatisfactory order is both revealed and concealed by the index. Certain words are printed in italic type. These, as is usual, include specific names and those Latin phrases that are often spoken but no longer heard. Certain terms also are put in italics but only once when they are defined. In the case of " chiasma " the index admits three uses (pp. 63, 67 and 70) before the term is defined (p. 75). In the case of " bivalent ", however, the first index reference is to p. 74 where the word appears in italics but it is used earlier on p. 63. Its acknowledged use here became necessary because White deemed it wise to describe the diplotene lampbrush chromosomes of the Amphibia before giving his account of meiosis. The dust cover is proud of this account. It claims that " here *at last* (the italics in this case are mine) is a work which deals with meiosis comprehensively—the more advanced aspects, which many students find difficult, have been kept separated in a different chapter " and separated, we may add, by a chapter on chromosome rearrangements.

What has White decided to treat separately? In his initial account he points out that pairing may begin near the chromosome ends. Here he deals also with chiasma interference, positive if not negative, but the localisation of chiasmata is considered separately as a special problem. Meiosis in polyploids is considered in the special chapter also and allotetraploids are described before diploid hybrids.

Between the two chapters dealing with meiosis comes, as we saw, one on chromosome rearrangements. Here a section on interchanges and one on whole-arm rearrangements are separated by two sections, one on duplications and deficiencies and another on the mitotic and meiotic behaviour of ring chromosomes. Meiosis in inversion and interchange heterozygotes

is not dealt with in any of these chapters. The treatment given the former is typical of the inexplicable order of the whole book. Somatic pairing in inversion heterozygotes is mentioned on p. 57. Inversion loop pairing at meiosis is considered on p. 72. Inversions are defined on p. 90 and then we have the inversions in " sex-ratio " *Drosophila* (p. 99), the effect of inversion heterozygosity on synapsis in species hybrids (p. 107), inversion differences between sex chromosomes (p. 119) and the conservation of inversion heterozygosity by parthenogenesis (p. 134). The reader has to wait until p. 139 before learning of the consequences of crossing-over in inversion loops.

A somewhat muddled order is perhaps forgivable in a first edition but not in a fifth. One can forgive also the half-truths which are inevitable in an elementary text. But there can be little justification for including comments which will have no significance for the intended reader. For example, fig. 14 shows two types of terminal chiasma. In one the paired chromatids are both maternal or both paternal, in the other maternal and paternal chromatids are paired on both sides of the chiasma. White points out that he is of the opinion that all terminal chiasma are of the second type though Darlington believes that both types are possible. Since the elementary reader is not aware, and is not made aware, of even the nature of the problem why present him with a difference of opinion which he cannot possibly appreciate ?

Again, we will all be pleased to know that " very complicated chromosome cycles have been described in the various races of the pseudogamous planarian *Dugesia benazzii* from the island of Sardinia". But since White has no intention of telling us what these cycles are, let alone what their significance may be, one wonders why he bothered to give us this gem of information.

In his concluding remarks White claims that " when we consider the varied types of life cycles and the many different kinds of population structure met with in plants and animals the great variety of chromosome mechanisms—begin to take on a meaning ". Agreed, but the reverse is true also for their selection is mutual. White (in this book at least) shows no appreciation of this as is evidenced by his up-side-down treatment of structural heterozygosity. The reader is left hanging " like a bat in a world of inverted values ".

Those who have the task of teaching the elements of genetics will appreciate White's difficulties but his method of tackling them they will not understand. The people for whom the book was intended will gather little from it for it is intelligible only to those who have no need of it.

K. R. Lewis.

THE EVOLUTION OF MAN. By G. W. Lasker. New York: Holt, Rinehart and Winston. 1961. Pp. 237. $3.50.
THE PROCESSES OF ONGOING HUMAN EVOLUTION. Ed. G. W. Lasker. Wayne State University Press, Detroit. 1961. Pp. 108. $3.75.
MOLECULAR GENETICS AND HUMAN DISEASE. Ed. L. I. Gardner. C. C. Thomas, Springfield, Illinois; Blackwell, Oxford. Pp. 297. 92s.

The first of these three works, an account of human evolution, is in two parts: a description of the fossil evidence and a statement of genetic problems connected with migration, selection and race formation. The materials are good but the connections between them are no clearer at the end than at the beginning.

If the author had assimilated something of Galton he would have made more of his subject. His serious omission, however, is that of the breeding behaviour of man. The fact that the social anthropologists have failed to understand the genetic importance of their work does not excuse us for neglecting to do so. Physical and social anthropology and genetics make sense together. But they fail when one is left out.

Symposia we expect to be disconnected but the first of these, or some of it, hangs together. Four of the six articles have a genuine connection with the adaptation of human races. Motulsky gives us a valuable account of the historical and geographical genetics of disease resistance, a study now so rapidly advancing. Only two references are lacking. The founder of the genetic theory of disease resistance, and indeed of race formation in man, is W. C. Wells whose essay, referred to by Darwin, was published in 1818. Its principal recent exponent was Archdall Reid in 1905. We ought not to forget the pioneers of this important problem.

Questions of selection are discussed by Hulse in relation to plasticity and heterosis. He points out a possible relation of mediæval serfdom and inbreeding to the supposed decline in height of European populations and this helps to show where our enquiries are leading us. The discussion, however, is inconclusive, inevitably so for it requires one dimension more than the author cares to admit.

We are given two accounts of where the authors of this symposium work but the two do not agree.

The symposium on Molecular Genetics and Human Disease contains one paper on the theory of molecular genetics, two connected with irradiation genetics, two on the theory of population genetics, three on abnormal chromosome complements in man; but only four deal with the actual subject proposed in the title. The problems of genetic susceptibility to infectious disease are here considered only in relation to malaria. These are of the most immediate interest. There is also a sketch of the history of genetic theory from Leonardo to Oparin illustrated with portraits entitled " Molecular Genetics and the Skein of Time ". A short glossary of genetic and cytological terms, presumably for chemists, concludes the work.

An introduction by Linus Pauling points out the eugenic problems raised by some of the papers in this symposium. Most of the papers contain matters of interest but nearly all are too condensed to be of general value. The verbatim remarks at the ends of papers, which are perhaps intended to supply continuity and connection, are not likely to satisfy anyone, even the authors.

C. D. DARLINGTON.

GENETIC RESEARCH. By A. Müntzing. Stockholm: LTs Förlag. 1961. Pp. 327. £2, 10s.

John Masefield once said that " in life not many have one chance and none has two ". Professor Müntzing, however, has had no less than three chances to present genetics in the form of a textbook. The first in his own native language, the second in a translation of this work into German and the third an English translation of the second Swedish edition. The Swedish and German texts appear to have been highly successful; this is not surprising since there is a drastic shortage of textbooks of genetics in the Scandinavian countries and in Germany. But such a shortage does not apply to the English-speaking countries. This is not to imply that *new*

books are unnecessary but it is to imply that such a book should either be different or at least should attempt to say familiar things in an unfamiliar or better way.

Unfortunately this is not true of *Genetic Research* for while the title is novel, though misleading, the contents are not. Thus one-fifth of the figures with which this book is illustrated are taken from existing textbooks written in the English language and some of these can certainly not be said to have been chosen for their lucidity. To this reviewer it is amazing that the author should have had to resort to using the mitotic illustrations of Buchner (fig. 9) and Wilson (fig. 10) to illustrate a process for which superlative preparations can now be made by students themselves. This is perhaps all the more surprising when one remembers that so much chromosome work has come out of Professor Müntzing's own department.

Not only is the approach of classical form, so are many of the errors. It is worth considering some of these; they fall into three groups—the arrangement of material, the inaccuracy of factual content and the superficiality of treatment.

Although " genetic research has developed from the foundations laid by Mendel " (p. 18) the layout of this book does not in fact do so. The account of Mendel's work is delayed until p. 39, despite the fact that information which appeared after Mendel, and actually depends on his findings, is preferred before him. Other peculiarities of arrangement include :

(1) " Extra-chromosome inheritance " coming between " polyploidy and other changes in chromosome number " and " species formation and evolution ".

(2) " Inbreeding degeneration and heterosis " bridging " The nature of genes " and " species hybrids ".

(3) The inclusion of " the inheritance of quantitative characters " after " meiosis and crossing-over " but completely separated from " Multiple alleles " which appears some three chapters later; the interval being taken-up with the sequence " hereditary adaptation ", " sex determination " and " sex linked inheritance ".

In a word, like so many other *basic* textbooks of genetics this consists of semi-isolated chapters with little connection and little *basic* arrangement.

Finally, in his first treatment of meiosis (p. 26) the process is dealt with without the *complication* of chiasma formation which subsequently appears as a *necessary complication* forty-one pages later. If it was felt necessary to simplify meiosis by separating the segregation of homologous chromosomes from crossing-over then surely this could have been better achieved by dealing initially with an organism like male *Drosophila* where the genotype rather than *convenience* determines absence of chiasmata.

When one turns to the factual content the situation is little improved. To take a few examples. On p. 121 we are told that " the occurrence of a ring of four chromosomes in an organism which otherwise forms only chromosome pairs is a reliable symptom of heterozygosity for a reciprocal translocation ". This is just not true—the presence of two extra homologous chromosomes can lead to the formation of a ring of four in that chromosome which is represented four times with bivalents only forming in the remainder. In the same way the presence of dicentric chromatid bridges and acentric

fragments is described on p. 124 as " a characteristic symptom of heterozygosity for inversions " despite the fact that there is now unequivocal evidence that bridges and fragments of this type can arise in other ways. Indeed the bridge and fragment in rye shown in fig. 87 is almost surely not due to inversion heterozygosity at all but rather to chromosome breakage and sister reunion of the type Rees and Thompson found in this species in 1955.

Another statement which may surprise the critical reader is that " the irradiation of the resting stage will lead to chromosome aberrations " while " irradiation of *prophase* stages, on the other hand, will lead to chromatid aberrations ". These and other errors are ones that are not confined to Professor Müntzing's book but they are less excusable in a work as recent as this, particularly since the author has had no less than three opportunities of remedying them.

Perhaps the most serious of the faults of this book is, however, its superficiality. Thus on p. 196 we are told that " a peculiarity of the inbred lines is that they often show characteristic disturbances of meiosis " but we are not told why; nor will the reader be able to discover this elsewhere in the book for there is no mention of genotypic control of chromosome behaviour.

Admittedly the book is wide in scope and most other aspects of cytology and genetics are covered, if only briefly. However, few questions are posed, few new horizons opened up. This may satisfy the casual reader, it cannot satisfy the true student. One is reminded of Harriet Monroe's lines

> " You dream the same old idle dreams
> You move not in the drift of years ".

When the German edition of this book was reviewed in *Heredity* there were, as Professor C. D. Darlington pointed out, no references. The English edition makes no attempt to remedy this though on p. 126 it does give what to me is the most surprising news of all " Håkansson made it clear that ring formation in *Œnothera* depends on translocation ".

Despite these criticisms I have little doubt that this book will sell both in this country and more especially in America, even at the rather high price of £2, 10s. My reasons for making this prediction are twofold. Firstly it is attractively produced and profusely illustrated and is sure to appeal to those who prefer to improve their bookshelves rather than their minds. Secondly, as Arthur Helps once put it " Reading is sometimes an ingenious device for avoiding thought ". For these reasons it may well join the ranks of the existing standard works in genetics. But it will never supersede them.

B. JOHN.

BOOKS RECEIVED

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ECOLOGICAL DISTRIBUTION OF GENETICAL VARIATION IN SOUTHERN SCOTLAND

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The data are derived from over 200 sites and consist of (*a*) ecological notes on the sites (floristics, altitude, rainfall, etc.) and (*b*) means for plant characters of populations derived from the sites after cultivation in trial. Analyses indicate that the relationships between the two groups of data are highly significant. Several species were isolated from each site and the interspecific correlations of plant characters are interesting. Thus sites which contained both *Potentilla erecta* and *Festuca ovina* (tetraploid) tended to have them in comparable sizes, *i.e.* both big or both little. It is argued that interspecific correlation of this type can only result from simultaneous selection within the two species by related environmental factors.

COMPETITIVE ABILITY OF NATURAL POPULATIONS OF *TRIFOLIUM REPENS* AND ITS RELATION TO DIFFERENTIAL RESPONSE TO SOIL FACTORS

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Recently attention has been drawn to the genetic determination of competitive ability of varieties of crop species, and the effect of soil factors upon this. Information concerning the response of natural populations, where response may be correlated with natural habitat conditions, would evidently be of interest.

Natural populations of *T. repens*, taken from contrasting soils, have been grown on those soils either separately or as pairs of contrasting populations. Differential response to the soils was found to be more marked under the competitive conditions of mixed planting, and most marked under field conditions of interspecific competition. The response of each population was closely related to its native soil conditions. The relative competitive ability of the various populations was found to be dependent upon the soil used; this might be regarded as genotype-environment interaction and is amenable to physiological analysis. Such analysis has shown that differences in response to calcium, magnesium, phosphate and iron are involved and that differential response to these nutrients is due mainly to differences in uptake.

The results throw light upon the physiological basis of population differentiation in response to soil factors and also lead to speculation on the role of competition in relation to natural selection.

EVOLUTION IN THE ROSTRATE VIOLETS—I

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The group (typical examples of which are the common dog-violets *V. riviniana and V. canina*) consists of some 30 species, and its total range covers the temperate regions of the whole northern hemisphere. Of the species so far counted, at least 13

are diploid, 6 are tetraploid and 2 hexaploid. Their relationships are being investigated both morphologically and by studies of meiosis in interspecific hybrids (*a*) between the European species and (*b*) between the European and N. American species. It is hoped eventually to include the Asiatic species, and to build up a picture of the evolutionary history of the group as a whole.

EVOLUTION IN THE ROSTRATE VIOLETS—II

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Meiotic studies of hybrids involving 15 species by the authors and by D. M. Moore have shown that the tetraploid and hexaploid species all behave as allopolyploids. It has been possible to interpret the relationships between the species in terms of their constituent genomes, some of which are referable to known diploid species while others are as yet unidentified. A close relationship between the genome of one diploid Eurasiatic species with that of the American diploids has been established. Some of the results from hybrids between the diploid species are, however, not consistent with a simple genomic interpretation and these will be discussed.

INTERCHANGE HETEROZYGOSITY IN *ANTHOXANTHUM*

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Cytological studies show that the condition of interchange heterozygosity known to occur in the tetraploid *Anthoxanthum odoratum* ($2n = 20$) is typical of the species as such. No homozygotes have been found in natural populations in Britain. It is postulated that the heterozygotes are not the product of gradual accumulation of interchanges but are the result of a hybrid origin. The F_1 hybrid between putative diploid ancestors, *A. alpinum* and *A. puellii* ($2n = 10$) is heterozygous for several large interchanges. Pairing between the sets of these species is continued in their artificial amphidiploid which both cytologically and morphologically resembles *A. odoratum*. Disjunctional orientation of multivalents controls the segregation of the parents' sets, one of each of which passes to each gamete. Zygotes are thus alike in their basic constitution and all will be heterozygous for interchanges.

THE EVOLUTION OF A PATTERN IN *DROSOPHILA*

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Continued selection for an increase in the number of bristles and ocelli in the *ocelli-less* mutant of *Drosophila subobscura*, which is variable for the number of bristles and ocelli on top of the head, has resulted in the appearance of a new pair of bristles. These bristles always arise at a specific site and have a definite orientation. The "neomorphs" are normally absent in the Drosophilidæ but they are present in a family closely related to it, namely the Aulacigastridæ.

A hypothesis is postulated to explain the origin of neomorphs in terms of an unvarying "prepattern" which determines the positions of these structures, and of a common "precursor" of bristles and ocelli which must be present in the required amount if structures are to be formed. It is suggested that if the amount of the precursor is increased to a certain threshold, a new bristle is formed in response to a peak of the prepattern which is present in the wild-type flies, but to which wild-type cells are not competent to respond.

NON-RANDOMNESS OF MITOTIC RECOMBINATION IN YEAST

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Mitotic recombination allows homozygosis at loci previously heterozygous and for any given locus this will occur spontaneously with a certain frequency. This frequency can be increased considerably by ultraviolet radiation. If the event is a random one, simultaneous homozygosis at two or more loci will occur at a rate equal to the product of their separate probabilities. In strains heterozygous for a number of genes, multiple homozygotes occurred at a rate too high to be consistent with this hypothesis. It is suggested that the process takes place between all homologous chromosomes in a dividing nucleus when a certain set of conditions obtains. That homozygosis did not result from non-disjunction or partial haploidisation was shown by including two sets of linked markers in the strains used; those that became homozygous at one locus were not generally homozygous at the linked locus.

FERTILITY AND CHROMOSOME PAIRING IN AUTOTETRAPLOID RYE

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Fertility in autotetraploids depends in part on the frequency and distribution of multivalents and other chromosome configurations at meiosis. These frequencies and distributions in turn depend on the chiasma frequencies—as is shown in a recent theoretical model presented by Durrant (1960). Since chiasma frequencies are known to be under genotype control one should expect heritable variation in the distribution of multivalents and hence in the fertility of autotetraploids.

The work described confirms such variation among autotetraploids produced from F_2 families in rye. Evidence is also produced showing that heritable variation in the distribution pattern of multivalents at meiosis, and hence in plant fertility, can be independent of chiasma frequencies. Finally from the standpoint of breeding for high fertility in tetraploid rye our evidence shows that the most effective selection would be for high quadrivalent frequencies.

PARTITION OF THE D GENOME IN *TRITICUM*

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Partition of genome D of *Triticum æstivum* by analysis of the genetic effects of individual D chromosomes on a common background constituted by genomes AB of *T. durum* requires some knowledge of the relative frequencies of aneuploid and euploid gametes from pentaploid hybrids.

A comparison of the direct and indirect estimates of the frequencies of male and female gametes from such hybrids indicates that certation is the main cause of the elimination of some chromosomal types from the selfed and backcross progenies. Not only are euploid pollen grains much more successful than aneuploid, there being an inverse relationship between success and degree of aneuploids, but there is specific complementation between pollen grains and styles having identical genomic constitutions. These results together with the marked instability of the additional chromosomes in haplosomics and diplosomics suggest that each of the genomes of this established allopolyploid still functions as an integrated unit.

A re-appraisal of the methods of partition analysis and alien chromosome addition studies shows that the genomic pattern of the allopolyploid series within the genus *Triticum* permits two types of partition analysis of the genomes in *T. æstivum*.

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